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THE EFFECT OF MIXING AND FERMENTATION UPON THE PROTEIN STRUCTURE AND COLLOIDAL PROPERTIES OF DOUGHS[1]

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Decided alterations in the physical properties of bread doughs may be effected by overtreatment in a high-speed mixer. A firm, dry, coherent, elastic mass may be changed in a few minutes to one having properties of softness, stickiness and lack of tensile strength to such a degree that it can scarcely be handled without a spoon or spatula. It might not be unreasonable to suspect that some alteration in chemical composition would accompany such drastic changes in colloidal properties. Chemical tests of normally mixed and fermented doughs have not effectively disclosed any substantial decomposition or other chemical alteration of the flour proteins, nor does the literature reveal any studies of such possible changes in overmixed doughs.

The increasingly common practice of adding skim-milk solids to bread doughs has created new problems in dough behavior. Properly prepared dry skim-milks impart excellent qualities of firmness and stability to bread doughs. Dried skim-milks prepared with insufficient heat treatment have appeared to accelerate the physical breakdown of any dough in which they have been incorporated when subjected to extremes of mixing or fermentation.

In this investigation an attempt was made to obtain additional information with regard to the physico-chemical changes that occur during the mixing and fermentation of bread doughs. Methods previously

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employed for determining changes in the percentage of primary proteins were applied to normally mixed and fermented doughs in verification of the results of other investigations. Such methods were also applied to doughs that had been decidedly overmixed and to doughs containing dried skim-milk and fluid skim-milk of variable baking quality. In some cases, proteases were added to accelerate the rate of protein decomposition. Tests of physical dough stability during mixing were made by the Farinograph for comparison with observed changes in chemical composition of the dough proteins.

Review of Literature

Mention will only be made of outstanding investigations that have a direct bearing upon the studies herein reported. The monumental work of Osborne and co-workers included studies of the utility of copper hydroxide as a protein precipitant. Precipitation was desired primarily to obtain the native proteins in order to study characteristics and amino acid distribution. However, the methods used by Osborne and Leavenworth (1916) in effecting a protein precipitation with copper hydroxide have served as a basis for many later investigations.

Blish (1918) used several protein precipitants in studying the so-called non-protein nitrogen in wheat flour suspensions. The amount of non-protein nitrogen obtained was found to depend upon the precipitant, being least when copper hydroxide was used. Methods for copper hydroxide precipitation of proteins from flour suspensions were extensively studied and developed by Blish. Olsen and Bailey (1925) used copper and tin precipitation methods for determining proteolysis caused by the addition of yeast to flour suspensions. The amount of non-precipitable nitrogen increased very slowly with extended fermentation. Cairns and Bailey (1928) compared eight methods for determining proteolytic activity in flour suspensions. Of the precipitants used copper hydroxide and tungstic acid were found to give the most consistent results. Digestion for as long as 48 hours gave evidence of relatively small increases in the amount of nitrogen not precipitated by the various reagents.

Johnson and Bailey (1924) studied the changes occurring in cracker doughs during extended fermentation. They found definite evidence of proteolytic activity in fermenting doughs but at a very slow rate during the first hours of fermentation. Brownlee and Bailey (1930) used several chemical methods for detecting proteolytic activity in normal bread doughs. The doughs were inactivated by freezing, then ground and dehydrated in acetone. The various methods used gave little evidence of change in protein composition during a five-hour fermentation.

The rate of formation of non-protein nitrogen was accelerated by the addition of proteases.

There is a generally recognized inadequacy of existing methods for determining the amount of protein decomposition. Bailey (1925) says that " it is conceivable that substantial modification of the gluten proteins in contact with active proteases may result without any material increase in the simpler degradation products of proteolysis which can be estimated by analytical methods now available." The wide discrepancy in the amount of nitrogen not precipitated by the various recognized protein precipitants indicates that the nitrogenous material in flour exists in groups of various sizes and that protein decomposition does not immediately result in groups that even approach uniformity in dimension. Thus the use of copper hydroxide as a precipitant removes a larger percentage of the nitrogenous material at any stage in the decomposition than most other precipitants, due to its greater efficiency in flocculating the smaller polypetides. It is apparent that one cannot determine, with present methods, the proportion of protein and peptide fractions that appear during the fermentation of doughs. Only a crude measure of the rate of such change can be obtained if the assumption is justified that the rate of division into small enough groupings to be non-precipitable can be used to indicate the extent of possible cleavage into larger units. It was thought that possibly changes in protein aggregation occurring during excessive mixing might be great enough to be measurable by precipitation methods.

Precision has been added to studies of the physical changes occurring in dough during mixing by the use of a device known as the Farinograph. This apparatus for accurately measuring the changes in dough plasticity during mixing has recently received considerable attention in both American and European cereal laboratories. Descriptions of the apparatus and attempted applications have been reported by Skovholt and Bailey (1932), Brabender (1932), Katz (1934), and Landis (1935).

The Farinograph is especially useful in determining the effect of different qualities of dry skim-milk upon the colloidal properties of dough when subjected to mechanical mixing. It also discloses the effect of small additions of proteolytic enzymes upon the same dough properties. A marked similarity has been observed in the plasticity curves of doughs containing dry skim-milk of inferior baking quality and of doughs containing small amounts of added protease.

The application of heat to fluid milk before drying invariably improves the baking quality of the product. The conditions of temperature and time that are most effective in causing this improvement are essentially the same as those required for lactalbumin coagulation. The

striking similarity in properties exhibited by proteins such as albumin and peptic enzymes has been pointed out by Northrop (1930). The possibility that the improvement in baking quality effected by heat treatment may be due to enzyme inactivation, is thus suggested. It has also been shown by Tarr (1934) that the heat resistant spores of bacterial organisms may elaborate proteolytic enzymes following a heat treatment that would be severe enough to cause enzyme inactivation. It was thought that possibly protein precipitation methods might throw some light on the nature of the effects of dry skim-milks upon dough properties. Doughs containing " good " and " poor " dry skim-milks as well as some with added protease were included, therefore, in studies of protein decomposition during extended mixing and during fermentation.

Experimental

Methods

The method used for the study in bread doughs of the nitrogen not precipitable by copper hydroxide, was essentially that of Johnson and Bailey (1924) except for modifications in size of aliquots taken and method of dough disintegration. A dough aliquot equivalent to 50 g. of flour was divided into small pieces and placed in a wide-mouth bottle containing about 100 g. of water. This was subjected to the vigorous agitation of a high speed " malted milk " mixer for three minutes. This treatment was quite effective in creating a smooth, lump-free suspension in even less time than the three minutes allowed. The suspension was then rinsed into a larger flask and made up to 300 g. Two or three fifty-gram aliquots of this suspension were then placed in small Erlenmeyer flasks and 20 cc. of 0.2 N NaOH added to each. Sufficient 0.2 N $CuSO_4$ was added to each sample to cause the color to change to a decided green using a few drops of phenolphthalein in each aliquot. The amount of $CuSO_4$ required was about 21 cc. To aid in the protein precipitation the samples were shaken vigorously several times during the period of about an hour usually allowed for flocculation. The aliquots were then made up to 100 g. and centrifuged for 10 minutes at a speed of 2500–3000 r.p.m. The suspensions were filtered and 60 g. of filtrate taken for nitrogen determinations. This weight of filtrate represents one-tenth of the total sample and was therefore obtained from dough equivalent to 5 g. of flour.

Tungstic acid was also used as a protein precipitant in another series of determinations. The weights of aliquots taken and the method of dough disintegration was the same as with the copper hydroxide precipitant. To each 50 g. aliquot (representing one-sixth of the original 50 g. flour dough), 3 cc. of 15% sodium tungstate was added and acid-

ulated to a distinct pink with concentrated sulphuric acid using thymol-blue as indicator, then adding an excess of 2–3 drops. These mixtures were then repeatedly shaken and centrifuged in the manner already outlined. Nitrogen determinations were made on the filtrates from both precipitation methods by the Kjeldahl-Gunning method.

Lack of agreement between replicates in some of the first determinations was ascribed to incomplete protein precipitation as evidenced by turbidity or opalescence in the filtrate. Closer attention to the details of the technic as outlined eliminated many of the irregularities. Water-clear filtrates were not always obtained but the effect of a slight opalescence could not be detected in comparing the notations made of the clarity of each filtrate with the obtained nitrogen value.

Materials Used

Only one flour was used in the protein precipitation studies. This flour (No. 15470) was a family patent of moderate baking strength and was milled from a mixture of hard spring and hard winter wheats. It contained 11.2% of crude protein ($N \times 5.7$) on the moisture basis of the sample when used.

Two dry skim-milks were used in several doughs, in the recommended concentration of 6% based on flour weight. Dry skim-milk No. 3123 was known to be of poor baking quality as shown by repeated baking and Farinograph tests. Dry skim-milk No. 3124 was of good quality from a baking standpoint. Eli Lilly's granular U.S.P. pepsin and trypsin obtained from the Digestive Ferments Company were added in small amounts to certain of the doughs produced. In one series of comparisons, fluid skim-milk was obtained fresh from the University Farm creamery and divided into two portions. One lot was added to doughs without treatment and the second lot was heated to 80° C. for 30 minutes with the flask connected to a reflux condenser to avoid concentration of the milk before using in dough preparation. The efficiency of such a heat treatment before drying in improving baking quality has been demonstrated by Greenbank, Steinbarger, Deysher, and Holm (1927), Grewe and Holm (1928), and Skovholt and Bailey (1931). That such a treatment is equally effective when using the undried fluid milks in bread doughs has been frequently demonstrated by the authors and is indicated in a publication (1932) previously cited.

Results

In studying the use of copper hydroxide as a protein precipitant, determinations were first made of the non-precipitable nitrogen from doughs that had been mixed for 1 minute and 10 minutes, respectively.

in a Fleischman-Bachman laboratory mixer. Mixing on this machine for 1 minute results in a dough exhibiting little development and gives little more agitation than necessary to obtain a uniform distribution of ingredients. A 10-minute mixing is sufficient to cause a decided physical breakdown in doughs made from average flours. After largely eliminating errors due to faulty technic it became apparent that this precipitation method did not reveal any appreciable increase in non-precipitable nitrogen due to this mechanical treatment of doughs. The values obtained for non-precipitable nitrogen, expressed as milligrams of N per 10 grams of flour are listed in Table I.

TABLE I

EFFECT OF EXCESSIVE MIXING OF DOUGHS UPON THE AMOUNT OF NITROGEN NOT PRECIPITATED BY COPPER HYDROXIDE

	Mixed 1 minute	Mixed 10 minutes
	Milligrams N per 10 g. flour	
Dough without milk solids	5.1	6.1
Dough with 6% D.S.M. No. 3123	8.6	9.0

The amount of increase in nitrogen compounds not precipitable by copper hydroxide by this excessive mixing was small in both dough types. It was even less in the dough containing the poor quality dry skim-milk than in the milk-free dough in spite of the evidently greater physical breakdown in the former type.

Comparisons were also made of the amount of increase in nitrogenous compounds not precipitable by copper hydroxide after extended fermentation of doughs without milk solids and of doughs containing " good " and " poor " quality dry skim-milk. Normal amounts of sugar, salt, and yeast were included in these doughs which were given a fairly vigorous mixing treatment as effected by allowing two minutes time in a Hobart-Swanson mixer. Duplicate experiments were made to determine the amount of non-precipitable nitrogen immediately after mixing and after 44 hours fermentation. Average results are recorded in Table II.

TABLE II

EFFECT OF FERMENTATION OF DOUGHS UPON THE AMOUNT OF NITROGEN NOT PRECIPITATED BY COPPER HYDROXIDE

	Immediately after mixing			After 44 hrs. fermentation		
Experiment number	1	2	Average	1	2	Average
	Milligrams N per 10 g. flour					
No milk solids	7.5	7.6	7.6	8.1	7.7	7.9
6% D.S.M. No. 3123 (poor quality)	10.7	10.4	10.6	12.6	13.2	12.9
6% D.S.M. No. 3124 (good quality)	10.1	9.7	9.9	12.1	12.7	12.4

The higher initial values for doughs with dry skim-milk are undoubtedly due to the nitrogen in the milk solids. The slightly greater increase in non-precipitable nitrogen with fermentation of doughs containing milk solids may also be explained by a freeing of nitrogen from this source rather than from any breakdown of flour proteins. This seems reasonable when it is observed that the two dough types containing milk were almost identical in increased nitrogen though the doughs with dry skim-milk No. 3123 gave evidence of decided physical breakdown while those with dry skim-milk No. 3124 were little effected by these treatments.

Tungstic acid was used as the preciptant in several comparisons to obtain confirmation of results already cited. Three experiments were conducted using doughs of the composition previously described. These doughs were given a normal mixing treatment by being allowed three minutes in the Fleischman-Bachman mixer. Aliquots were then taken for determination of non-precipitable nitrogen and the remainder placed in a fermentation cabinet at 30° C. Similar determinations were made on the doughs after 22 and after 44 hours of fermentation. Average results of determinations that had been replicated six times are given in Table III.

TABLE III

INCREASE IN NON-PRECIPITABLE NITROGEN DURING FERMENTATION OF DOUGHS WHEN USING TUNGSTIC ACID AS PRECIPITANT

	Not fermented	Fermented 22 hrs.	Fermented 44 hrs.
	Milligrams N per 10 g. flour		
Milk free dough	3.8	3.9	4.3
Dough with 6% D.S.M. No. 3123	6.1	10.2	12.8
Dough with 6% D.S.M. No. 3124	5.2	9.9	12.8

A comparison of Tables II and III reveals that less nitrogen was found to be non-precipitable by tungstic acid than by copper hydroxide from all doughs not containing milk solids and from unfermented doughs containing dry skim-milk. After fermentation of the doughs containing skim-milk solids the values obtained by the use of the two precipitants are approximately equal. Apparently fermentation breaks down or releases the nitrogenous compounds of milk to such an extent that equal amounts of nitrogen are precipitated by tungstic acid and copper hydroxide from such systems.

Determinations were made of the non-precipitable nitrogen in 0.6 g. of each of the two dried skim-milks. This is the amount associated with 10 g. of flour in the doughs previously studied. Calculations were made to this basis since nitrogen has been reported as milligrams per 10 g. flour. Average values gave 2.7 mgms. non-precipitable nitrogen in 0.6

g. dry skim-milk No. 3123 and *1.4* mgms. in the same weight of dry skim-milk No. 3124. These values account almost exactly for the differences found in the amount of non-precipitable nitrogen in the unfermented doughs without milk and with the two dry skim-milks as given in Table III. It is likely that the difference in the amount of non-precipitable nitrogen as found in the two milks and as found in doughs containing these milks, when using either copper hydroxide or tungstic acid as the precipitant, is due to the fact that the fluid milk used in preparing dry skim-milk No. 3124 had received a higher heat treatment before drying than that dried to produce No. 3123. Such heat treatments as frequently used are known to be sufficient for partial coagulation of lactalbumin thus probably causing this nitrogenous material to be more easily flocculated by protein precipitants.

The results as listed in Table III do not show any appreciable difference in the extent of protein hydrolysis in the doughs containing the two dry skim-milks. In fact, the indicated increase in non-precipitable nitrogen during fermentation is slightly greater in the relatively firm doughs containing dry skim-milk No. 3124 than in the doughs containing dry skim-milk No. 3123, which showed decidedly greater evidence of physical breakdown.

Physical breakdown of dough is indicated by an increasing mobility during extended mixing in the Farinograph mixing bowl. The changes in force required to rotate the mixer blades through the dough are accurately determined by a dynamometer attachment to the shaft between the motor and mixing bowl and are indicated on a large scale and also recorded on paper moved by a kimograph. This graph paper is ruled to read in Farinograph units which need not be calibrated into absolute units to be of value for comparative purposes. Such Farinograph charts show a more or less rapid decrease in mobility during the early stages of mixing while ingredients are being incorporated into an homogeneous mass and during the so-called "dough development." Following the attainment of a plasticity peak, doughs gradually increase in mobility, the rate of increase being quite variable depending on the ingredients used. Flours are known to differ widely in their qualities for forming doughs that resist mobility increase during mixing. Dry skim-milk also impart widely different stability qualities to bread doughs. These differences in imparted stability as indicated by the Farinograph have been shown by Skovholt and Bailey (1932) to be a reliable measure of the utility of dry skim-milks in baking.

The qualities of stability during mixing that 6% of dry skim-milks No. 3123 and No. 3124 impart to doughs are shown in Figure 1. These doughs contain only flour, water, dry skim-milk and 1.75% common

salt. Included in this figure are also two curves obtained with the Farinograph on doughs which contained flour, water, salt, 6% dry skim-milk No. 3124 and 0.4% pepsin and 0.05% trypsin, respectively, based on flour weight. These enzyme preparations were dissolved in the water that was added at the start of the mixing operation. These curves were selected from a large number that were made with varying

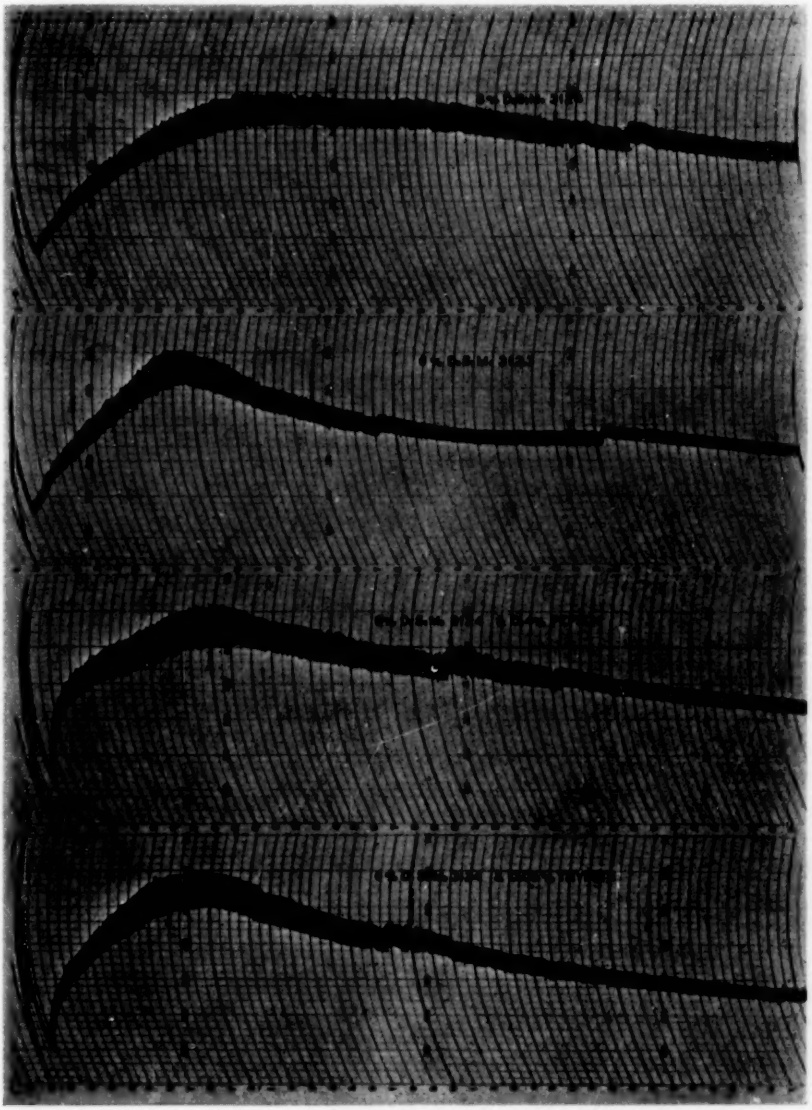

Fig. 1. The effect of dry skim-milks and proteolytic enzymes upon the mobility of doughs during mixing as recorded by the Farinograph.

concentrations of enzymes. They are shown since they indicate the similarity in mobility changes during mixing obtained on the dough containing dry skim-milk No. 3123 without enzyme addition with the doughs containing dry skim-milk No. 3124 plus proteases. The dosage of 0.05% trypsin was about sufficient to impart to doughs containing 6% dry skim-milk No. 3124 the same low resistance to mobility increase during mixing shown by dry skim-milk No. 3123 without addition of proteolytic enzymes. The use of 0.4% pepsin with dry skim-milk No. 3124 did not break down the dough quite as much as dry skim-milk No. 3123 alone when judged by mobility changes during a 40-minute mixing period.

Determinations of nitrogen not precipitable by tungstic acid were made on these four doughs immediately following the 40-minute mixing period. The results are given in Table IV.

TABLE IV

NITROGEN NOT PRECIPITATED BY TUNGSTIC ACID FROM DOUGHS MIXED FOR 40 MINUTES IN THE FARINOGRAPH

	Milligrams N per 10 g. flour
Dough with 6% D.S.M. No. 3123	9.8
Dough with 6% D.S.M. No. 3124	9.4
Dough with 6% D.S.M. No. 3124 + 0.05% trypsin	12.8
Dough with 6% D.S.M. No. 3124 + 0.4% pepsin	27.2

It is evident that the addition of proteolytic enzymes increases the amount of non-precipitable nitrogen even when determined immediately after a 40-minute mixing period. The value is especially high for the dough containing pepsin even though the Farinograph indicated a lower mobility for this dough at this stage than for the one containing 6% of dry skim-milk No. 3123.

Several doughs were mixed for 40 minutes with the Farinograph mixer in which salt, sugar, and dry skim-milk had been included. Yeast was also an ingredient in three of the four doughs and trypsin was included in one. Determinations of non-precipitable nitrogen were made immediately after mixing and after 5 and 24 hours of fermentation. Results are given in Table V.

TABLE V

NON-PRECIPITABLE NITROGEN IN DOUGHS AFTER MIXING AND FERMENTATION WITH TUNGSTIC ACID AS PRECIPITANT—*Results as milligrams nitrogen per 10 g. flour*

Fermentation time	0 hrs.	5 hrs.	24 hrs.
D.S.M. No. 3123—Sugar-salt-yeast	9.9	9.2	13.4
D.S.M. No. 3124—Sugar-salt-yeast—0.05% trypsin	12.7	13.7	23.7
D.S.M. No. 3124—Sugar-salt-yeast	6.9	6.7	11.4
D.S.M. No. 3124—Sugar-salt (No yeast)	7.7	7.9	10.2

This series gave additional confirmation of the results given in Table IV though the values obtained with dry skim-milk No. 3124, without protease, were somewhat lower. Again there is no evidence of activity by dry skim-milk No. 3123 that approximates that of added proteases. This is most apparent in the results obtained after fermentation.

The results of Table V indicate a possibility of a consumption by yeast of nitrogenous material sufficient to show a decrease in two cases in the amount of non-precipitable nitrogen after the five-hour fermentation period. When measured after longer fermentation periods it is likely that ordinary yeast may accelerate proteolysis in doughs to more than overcome any nitrogen disappearance caused by yeast metabolism requirements. Definite evidence of such nitrogen consumption has not been secured by most previous investigators in this field. However, Samuel (1934) has pointed out that proteolysis studies as measured by amino acid production are made more difficult when yeast is used in flour suspensions due to a comsumption of amino acids by the yeast cells. The decreases apparent in the data of Table V may be due to this yeast consumption.

Only one series of duplicate experiments was conducted to determine the effect of extended vigorous mixing upon the amount of nitrogen not precipitated by tungstic acid. These doughs contained only flour, water, and 6% dry skim-milk No. 3123. Averaging all results, a value of *5.4* mgms. non-precipitable nitrogen per 10 g. of flour was obtained when these doughs were mixed for 1 minute on a Fleischman-Bachman mixer. When the same doughs had been mixed for 10 minutes *7.8* mgms. nitrogen were found in the filtrate per 10 g. of flour. This increase is somewhat greater than that obtained when using copper hydroxide as precipitant but the vigorous mixing may have released some of the nitrogenous material of the milk solids to account for the increase in the same manner as shown in the comparisons of the effect of fermentation. At any rate, the increase in non-precipitable nitrogen is too small to be commensurate with the decided physical breakdown shown in these doughs by this mixing treatment.

One comparison was made of the effect of heated and unheated fluid skim-milks upon the plasticity curve during the mixing of doughs and upon the amount of non-precipitable nitrogen from such doughs. Plasticity curves as obtained with the Farinograph on doughs containing these milks are shown in Figure 2. The effect of heating the fluid milk to 80° C. for 30 minutes in increasing the resistance of the dough in which it is included, to mobility increase during mixing, is clearly shown.

Triplicate determinations of the amount of nitrogen not precipitated

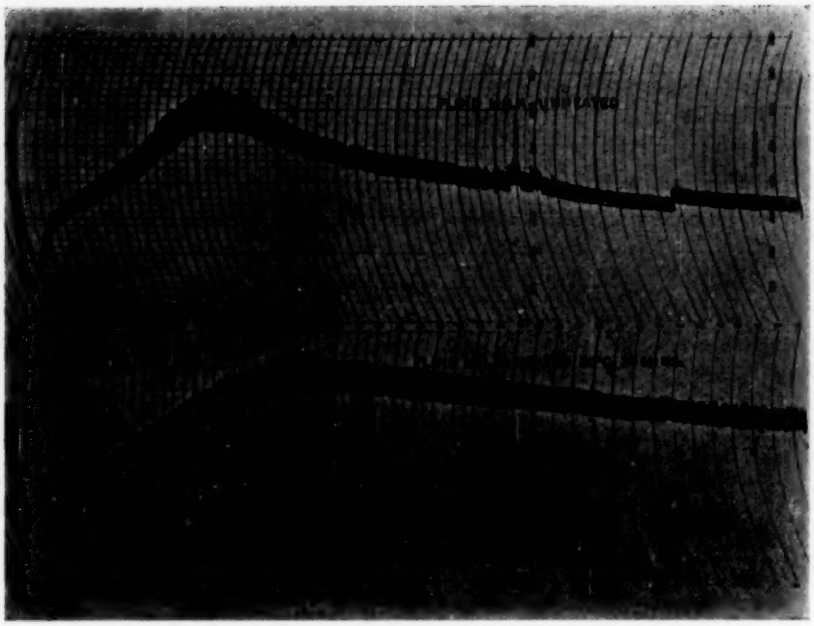

Fig. 2. The effect of heated and unheated fluid milks upon the mobility of doughs during mixing as recorded by the Farinograph.

by tungstic acid were made from each dough containing the heated and unheated milk, immediately after mixing and after 44 hours of dough fermentation. Doughs were prepared with sugar, salt, yeast, flour, and fluid milk. Averaged results are given in Table VI.

TABLE VI

NON-PRECIPITABLE NITROGEN AFTER MIXING AND FERMENTATION OF DOUGHS CONTAINING FLUID MILK USING TUNGSTIC ACID AS PRECIPITANT

	Milligrams N per 10 g. flour
Not fermented	
Unheated fluid milk	7.3
Heated fluid milk	6.8
Fermented 44 hours	
Unheated fluid milk	16.1
Heated fluid milk	15.1

As with the doughs containing dry skim-milk, there is no evidence with fluid milks of any greater increase in non-precipitable nitrogen when using unheated instead of heated milks to account for the observed difference in rate of dough breakdown during mixing and fermentation. There is also a difference in the amount of non-precipitable nitrogen from the doughs containing the two milk types that can be explained on the basis of partial heat coagulation of milk proteins.

None of the results that have been reported give evidence of protein decomposition of sufficient magnitude to account for the drastic differences in physical dough properties that are caused by extended mixing. Division of protein aggregates may occur and be partially responsible for the breakdown of doughs but little positive evidence of such division has been obtained in this work or that reported by previous investigators. There are indications of only a slight increase in non-precipitable nitrogen with fairly extended mixing and fermentation treatments even when using doughs of very unstable properties. Since the addition of proteases speeds up the rate of decomposition as determined by this index it can be assumed that the method is of value as a measure of protein decomposition. The results obtained by this method do not explain the reason for the drastic modifications in dough qualities effected by excessive mixing.

Summary

Nitrogen not precipitated by copper hydroxide or tungstic acid was only slightly increased by excessive mixing or by extended fermentation of doughs of ordinary composition. An appreciable increase in non-precipitable nitrogen was observed when protease preparations were added to the dough.

Certain milk samples seemingly exerted an effect upon dough properties during mixing and fermentation that was similar to the action resulting from the introduction of minute quantities of proteases. There was no increase in non-precipitable nitrogen effected by these milks, however, such as resulted from the inclusion of the proteases.

Proteins of heated fluid milks and of preheated dried milks appeared to be more effectively precipitated by cupric hydroxide or tungstic acid than those to which less heat had been applied, probably because a partial coagulation of nitrogenous constituents by heat made the precipitation by chemicals more complete.

Non-precipitable nitrogen cannot be used as the sole criterion of the hydrolytic or other chemical changes in protein aggregates or molecules in dough. Modifications of the physical properties of dough involving the gluten complex may occur even though the conventional biochemical methods fail to disclose appreciable quantities of those protein split-products which are differentiated by such means.

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FREE AND BOUND WATER IN BREAD DOUGHS [1]

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In the preceding paper (Skovholt and Bailey, 1935) it was shown that no evidence of chemical decomposition of proteins could be secured during excessive mixing of bread doughs. From superficial appearances such overmixed doughs appear to differ decidedly in ratio of water to flour when compared with the firm systems obtained after an optimum amount of mixing. The additional mixing causes a slackness that gives every indication of a lowered total solids concentration, although the total moisture content has not been altered. This might lead to speculation as to whether or not the water is held with equal firmness in doughs of such widely differing consistencies.

Water imbibition plays a prominent rôle in all stages of dough preparation. Thus dough is itself formed by the hydration of the dry flour particles. A proper mixing of doughs also involves a so-called development which may mean principally the formation of a complete gluten network of thin strands. During this process there is an apparent imbibition of water as evidenced by a decreasing dough mobility. If mixing is continued beyond this stage a reversal is noted in this characteristic. Apparently, water-holding capacity is being reduced and the dough is being "broken down" from a firm mass to one having properties of slackness, stickiness, and lack of coherence.

Even though mixing is continued only to the optimum point, unfavorable changes sometimes occur in doughs during the necessary fermentation period. Doughs properly made from good quality ingredients maintain an optimum mobility during fermentation. Serious difficulties may arise when a baker finds that doughs are "slackening off" during fermentation. Aside from a deleterious effect which such a change generally has upon the resulting bread, is the problem of manipulation of such doughs especially in those modern shops using machinery for the dividing, rounding, and making-up of loaves from the dough. Thus the

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apparent changes in water imbibition of bread doughs during mixing and fermentation are of tremendous practical as well as theoretical interest.

Review of Literature

At present there is some disagreement with regard to the manner in which water is held in hydrophilic colloidal systems. The work of Nelson and Hulett (1920) demonstrated that methods for determination of moisture in hydrophilic colloids are entirely empirical and do not give a measure of total water present. They found that the moisture content of flour was considerably greater as determined by their more drastic treatment for water removal than that obtained by official methods.

Wo. Ostwald (1921) classified water retention of peat into five kinds, namely, (1) occluded water, (2) capillary water, (3) colloid water, (4) water retained osmotically, and (5) chemically bound water. Different and increasingly drastic treatment is required to remove the water held in these various ways in the order given. While agreement with this classification may not be universal, it is generally recognized that all the water of a hydrophilic colloidal system is not held with equal firmness.

Newton and Gortner (1922) in using a method that required the expressing of plant saps found a decided difference in the ease with which this sap could be expressed from different tissues. Their observations led to experimentation and a method for a differentiation of the water in a hydrophilic colloid into two kinds depending on the firmness with which the water is held by the colloidal material. This method is based on the experimentally indicated assumption that not all of the water in such a system is free to act as a solvent for materials that are normally water soluble. The method involves a determination of the freezing point of the colloidal system before and after the addition of a known amount of a solute, such as sucrose. If the freezing point depression due to this solute addition is greater than that expected from the calculated value, as based on the total water present, then it is assumed that not all of the water in the system (as determined by official moisture methods) is free to act as a solvent. The percentage of the total water not acting as a solvent can be readily calculated from freezing point data.

This fraction of the total water present, these authors have defined as "bound." Considerable controversy has arisen over the validity of the methods used and doubt has been cast by some investigators on the existence of bound water. Newton and Gortner's terminology agrees with that of Hill (1930) except that the latter has used direct vapor pressure measurements and substitutes the concept of vapor pressures

for freezing points. Hill says that " the free water fraction is the weight of water in one gram of fluid or tissue which can dissolve substances added to it with normal depression of the vapor pressure." Greenberg and Greenberg (1933) suggest that the above definition can be made more general by using the term " change in colligative properties " instead of " depression of vapor pressure." Obviously, with this concept of " free " water, " bound " water is the difference between the total and the " free " water present. This is the sense in which the term " bound water " has been used throughout the following discussion. Precise definition is essential as it is likely that there is no sharp point of demarkation between the " free " and the " bound " water of any system. Variations in method or minor technic may well shift the results when differentiating between the two. The failure to recognize that such methods make an arbitrary division in systems in which water may range from that very loosely to that very firmly held, may be the cause of some of the confusion and many of the disagreements encountered in the literature dealing with this subject.

Freezing point measurements were used by Newton and Gortner (1922) for the determination of bound water in its relation to winter hardiness in plants. Further work on this subject with the same technic has been reported by J. H. Martin (1927). The application of similar technic in studying the relationship of bound water to drought resistance has brought valuable results in the hands of Newton and W. M. Martin (1930).

Newton and Cook (1930) have attempted to determine if the bound water of wheat flour suspensions is an index of flour quality. Starches and proteins prepared from the flours were also studied separately. Results obtained by them will be discussed later in a comparison with the data of the present investigation. As far as is known this is the only reported attempt of the use of bound water technic in problems of baking quality. The investigations of Newton and Cook did not include a study of bread doughs.

Various solutes have been used in using freezing point technic for bound water determinations. Sucrose is probably open to the least objection of any that have been proposed for application in systems containing flour. Unfortunately, sucrose is believed to form a hydrate when placed in solution and the evidence at hand is not definite with regard to its degree of hydration. Scatchard (1921, and 1921a) found from studies of sucrose inversion and from vapor pressure measurements that the assumption of the formation of a hexahydrate best fits the experimental data when dealing with dilute solutions.

In the work of Newton and Gortner, the formation of a sucrose

hexahydrate was assumed in calculating the theoretical depression of the freezing point by sucrose. Thus the molecular constant for the depression of the freezing point was taken as 2.085° C. rather than 1.86° C. due to the increased molecular weight with hydrate formation. It was also assumed that a gram-molecule of sucrose when dissolved in 1000 g. of water would theoretically leave only 892 g. of free water due to sucrose hydration. This reduction in free water was corrected for when calculating bound water values from any depression in freezing point greater than the theoretical.

Grollman (1931) has indicated a disbelief in high bound water values and has criticized the data of Newton and Gortner on the ground that they did not take into account the effect which the removal of water by sucrose hydration would have on the freezing point depression caused by the dissolved materials in the original plant sap. He calculates bound water percentages from some of their data after making this correction and obtains lowered values. Grollman attempts to show that the results thus obtained are valueless as a measure of winter hardiness. Gortner and Gortner (1934) point out an unfortunate choice of data for Grollman's calculations and show that even with his correction the results are of value as indices of winter hardiness.

Sunderman (1932) reports an inability to find evidence of bound water in blood serum by freezing point technic. He defends the use of such technic and cites experimental data in support of a contention that the correction as called for by Grollman is not justified.

Some of the critics of freezing point technic for the determination of bound water have used other methods. A discussion of the rôle of water in living organisms with a review of methods in use for differentiating free and bound water has been given by Gortner (1929). Only outstanding recent work and investigations developing fundamental methods of other types will be briefly mentioned at this time.

Recently, Greenberg and Greenberg (1933) report that results obtained when using crystalloidal reference substance in a colloidal system followed by ultra-filtration and analysis of the filtrate failed to give evidence of appreciable bound water in several systems investigated. They claim that some of the results obtained by other methods are in error due to the failure of the investigator to appreciate the wide deviation in colligative properties of the system under consideration from those of an ideal solution.

Dumanski (1933) also used filtration technic. To his colloidal system sucrose was added as the reference substance, followed by a refractometric or polarimetric determination of the sugar in the filtrate. He obtained definite evidence of bound water in potatoes and sugar beets.

The highest values were found in boiled potatoes which had a water binding capacity of about 1.5 g. per gram of dry material.

Thoenes (1925), Robinson (1927) and others, have used calorimetric methods for determining bound water percentages. This technic is based on the assumption that bound water will not freeze at even — 20° C. and that therefore its percentage may be determined by measuring the amount of heat required to thaw the ice from a given weight of water in a system after allowing ample time for complete freezing at a low temperature. Robinson's correlations of known winter hardiness in insects with bound water determinations on such insects have added weight to the belief in the value of such methods. Jones and Gortner (1932) demonstrated high amounts of bound water in systems containing gelatin and egg white by using a dilatometric technic in following volume changes during the freezing of these systems to temperatures as low as — 50° C.

Lloyd (1933) and Lloyd and Phillips (1933) have contributed extensively to our theoretical knowledge of protein structure and its relation to hydration. They postulate two principal mechanisms by which water may be firmly held by proteins and other hydrophillic colloidal substances. The first mechanism is that of hydrate formation in which the O or H of water attaches to the protein by a coordinate or semi-polar link. Thus O has two pair of electrons not shared in co-valent linkages and these may be donated to the H atom of such groups as the — OH, — NH_2 and = NH of the protein molecule. Similarly, the two H atoms of water can accept electron pairs from the O and N atoms in the — OH, — NH_2, = NH and — COOH groups abundantly found in all proteins. Lloyd and Phillips think that there is abundant evidence for the belief that proteins form hydrates in the same manner as such soluble substances as the various crystalline salts, sucrose, etc. Such water held as a hydrate, formed by the coordination of a water molecule with an oxygen- or nitrogen-containing group in the protein molecule is called "water of imbibition."

Besides the water held by coordination at electrically neutral groups, there is also the water held at the charged centers of the molecule. Every ion in an aqueous solution has around it a shell of oriented water molecules held by the forces of electrical attraction. Some of the amino and carboxyl groups of the protein molecule exist in aqueous solution in a charged condition. By the recently proposed Zwitterion theory, the number of charged centers in a protein molecule when in aqueous solution is appreciably greater than was formerly postulated. Regardless of the theory accepted, it is known that charged centers are present when proteins are placed in water and there should be no doubt but that such centers hold oriented water molecules by electrical attraction.

If bound water is defined as that water " held either by coordinated links to definite chemical groupings or by electrostatic forces around charged centers," as proposed by Lloyd, then it is evident that no sharp distinction can be drawn between bound and free water, especially since there may be a difference in the firmness with which primary and secondary layers are held. The relative amount of free and bound water will depend upon the conditions in the system under consideration and the method used for differentiation. Any method that arbitrarily determines differences in water binding in different systems, when applied under fixed conditions, may be useful.

Freezing point methods have probably had the widest application in the study of water binding. Such a method was therefore used in this study which concerned itself principally with attempts to determine if differences in water holding forces were present in doughs made with different flours and after modifications of plasticity by mechanical mixing.

Experimental

In the investigation of Newton and Cook (1930) already referred to, determinations of bound water were made on suspensions of flour in water by a cryoscopic technic. Possibly there is a difference in the molecular configuration of the gluten proteins as existing in flour-water suspensions in comparison to that found in the gluten network of doughs. Any attempt at determining changes in water holding forces such as might occur in doughs during fermentation or as a result of mechanical mixing would have to be made on dough systems since no similar treatment of flour is possible without dough formation.

Apparatus and Methods

All known previous applications of cryoscopy for determining bound water have been made on fluid systems. Thus in studying plant tissues, the juice has been expressed and solid matter removed by centrifuging or filtering. In the study of flour suspensions, the suspended material was removed by centrifuging before making freezing point determinations on the decanted solution. Experiments with a hydraulic press indicated that a separation of solution or sol from bread doughs was extremely difficult or impossible. Consequently, it was necessary to devise a technic that would satisfactorily give a measure of the freezing point of a plastic mass such as bread dough.

The apparatus used for the freezing point determinations is shown in Figure 1. The thermometer was placed in the inner tube which was loaded with dough so as to be filled to a level well above the thermometer bulb. This tube was encircled by a wire coil stirrer and

was contained in a larger glass tube nearly filled with 20% sodium chloride solution. The stirrer could be manually operated in this salt solution thus providing a medium with reasonably uniform temperature surrounding the dough layer during the entire freezing operation. This apparatus was placed in a stone jar containing an ice-brine mixture

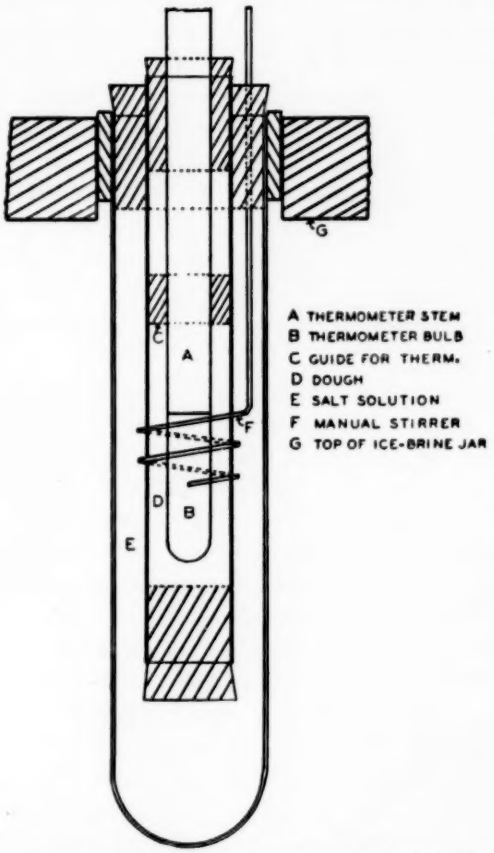

Fig. 1. Diagram of apparatus used for freezing point determinations.

which was kept in continual agitation during the freezing point determinations by means of a malted milk stirrer operated at moderate speed. The jar was fitted with a wooden top, one inch thick, with three bored holes through which the bath thermometer, stirrer and salt tube were inserted. Heat transfer to this freezing bath occurred rather slowly.

Early tests proved the necessity of having a well-centered thermometer bulb with the thin dough layer surrounding it of uniform thickness and entirely free from air pockets. This condition was difficult to attain in every trial when using an ordinary test tube as the dough container,

as was done in the first tests. Loading of the tube also required considerable time and the insertion of the thermometer required so much force when testing firm doughs that there was considerable danger of breakage.

These difficulties led to the adoption of open end tubes obtained by cutting off the test tubes previously used, near the closed end. One end of this tube was loaded with the proper amount of dough. The thermometer was then passed into the other end of this tube through a one-hole stopper and placed in the desired position while holding the thumb over the open end. The thermometer could then be held fairly well centered while inserting a tight fitting stopper thus forcing the dough into one compact mass to fill the tube to well above the thermometer bulb with no air pockets at any place in the layer between the thermometer bulb and the tube wall. A vent was made in the stopper holding the thermometer to allow the escape of air, thus avoiding difficulties incident to the building up of pressure when inserting the stopper in the lower end of the tube.

This procedure was used quite satisfactorily during an appreciable part of the investigation. Certain erratic results appeared from time to time that required additional checking. In some of these cases it was definitely noted that such errors were still due to poor centering of the thermometer bulb. They occurred especially when using doughs with high mobility and may have been caused not only by improper loading but sometimes by a shifting of the thermometer toward a wall of the tube during the progress of the determination. A well fitting cork guide was made, therefore, to rigidly hold the thermometer bulb in the center of the tube. It was placed on the thermometer some 50 mm. below the point at which it passed through the stopper. By a proper adjustment of the fits of the guide on the thermometer and in the tube, it was possible to get this guide and the thermometer into their proper positions in the tube. After some experience with this technic it was possible to complete the entire operation of removing the dough from the mixer, loading the tube and placing it in the freezing bath in an elapsed time of 1 to 2 minutes.

A Haidenhain thermometer with a range in readings from + 1.25° C. to — 5.30° C. was used throughout the investigation after a few preliminary tests. This thermometer was found to give a freezing point reading of + 0.115 in distilled water. This correction has been applied to all data before tabulation so as to give true freezing points rather than actual thermometer readings. The use of such a thermometer obviated the necessity for the frequent determinations of true zero point that would be essential with an unmodified Beckman in an ex-

tended investigation due to possible changes in the original setting. The Haidenhain thermometer was a little unsatisfactory in only one particular. Certain of the doughs containing high concentrations of added sugar were found to give an undercooling of sufficient magnitude to bring the mercury below the scale of the thermometer. In such cases, a scale drawn on paper was placed in back of the capillary and the low point in the undercooling could be approximately determined. The possible error incurred by such a procedure will be shown later to be insignfiicantly small in determinations of the type here attempted.

The importance of using the proper bath temperature was indicated early in the investigation. It was recognized that error is minimized by using a freezing bath as little below the freezing point of the system under investigation as is possible while still obtaining only a moderate degree of undercooling. In the systems here considered it was observed that the amount of undercooling was usually very great. The possible errors involved in correcting for undercooling are also recognized especially so in freezing point determinations on dough in which the specific heat of the system may vary appreciably from the value for water which is commonly used in making such corrections. When attempts were made to appreciably reduce the amount of undercooling obtained it was found to be necessary to use a bath temperature so low that serious errors might be introduced due to heat transfers in the system during the temperature rebound from the low point to the observed freezing point. Variable amounts of undercooling were also more evident when using an excessively cold bath.

Bath temperatures were standardized, therefore, at 5° to 7° C. below that of the observed freezing point in the particular system under consideration. It seemed desirable to use a slightly greater differential in temperature between bath and freezing point in those systems having a freezing point near zero than in those systems with a higher solute concentration. By thus slightly modifying the bath temperature to fit the system under consideration, it was possible to maintain a reasonable degree of uniformity in the amount of undercooling. Some irregularity was still apparent in occasional readings but the average undercooling for the different dough types was nearly the same as will be indicated later, and was of the order of 3.0° to 3.5° C.

The usual correction for undercooling was applied in all cases. One-eightieth of the difference between the low temperature during undercooling and the observed freezing point was multiplied by the observed freezing point and the product subtracted from the latter to give the corrected freezing point. It is recognized that this assumes that the specific heat of the dough system is the same as that of water.

While this is not strictly correct, the error incurred is not great and is uniformly applied to all dough systems so that the validity of comparisons is in no way involved.

Many preliminary determinations were made before standardizing on a method for the freezing procedure. As finally established, it involved repeated freezings of the same dough aliquot. Newton and Martin (1930) found that repeated freezings of plant saps led to erroneous values. Many replicated freezings and thawings of dough systems indicated no significant trend in successive values. Since dough may be appreciably altered if allowed to stand at normal temperatures, it was deemed essential to make all determinations that involved studies on the effect of mixing, immediately after the completion of mixing. If each aliquot could be frozen only once, it meant that a new dough would have to be prepared for each freezing point reading. Because of the observed variability in readings, considerable replication was considered to be essential. The procedure was adopted, therefore, of making a freezing point determination after which the tube containing the dough and thermometer was removed from the salt solution as soon as the temperature started to recede from the observed freezing point. This tube was then held in the palm of the hand until the temperature had again risen above the freezing point and allowed to stand about two minutes longer before returning it to the cooled salt solution for a second determination and this procedure was again repeated for a third reading. Because of the magnitude of the undercooling obtained, it was felt that it was desirable to avoid as much ice formation in the dough as might occur if the supercooled dough tube was not immediately warmed after ice crystal formation had begun and the hand provided a convenient and uniform source of heat to aid in preventing excessive rupturing of the dough structure. It will be shown later that these repeated freezings did not significantly alter the water imbibition capacity of the dough system.

The degree of agreement obtained in replicated determinations was decidedly lower than that expected from freezing point determinations on fluid systems. The study of each dough type in the major series involved determinations after 1, 2, 3, 4, and 10 minutes of mixing. The data show that the values obtained with each mixing treatment serve as confirmation of the correctness of other results obtained on doughs having been mixed for a different length of time in the same series. In the early part of the investigation, there seemed to be a marked irregularity of the effect of mixing in certain series. With the improved technic used later, these irregularities invariably disappeared when attempts were made to check the values obtained earlier. The

freezing point of any system was not considered to be established until duplicate doughs had been prepared and the triplicate readings as made on each dough were in fair agreement and showed a regularity in mean value with the determinations made on other doughs of the same composition but having received a different mixing treatment. Most of the series studied also involved determinations on doughs containing four different sugar concentrations so that additional confirmation of the validity of results was obtained in this way.

An erratic result could usually be predicted by abnormal temperature changes during the freezing operation. When the dough tube was properly loaded and the freezing bath at the correct temperature, the dough would be brought down to the low point in undercooling in some 6 or 8 minutes. When ice crystals began to form, a rapid rise in mercury ensued which gradually slowed down until a constant value was reached at the end of about two minutes. This observed freezing point value would be constant as apparent to the naked eye for a period of from 8 to 10 to more than 30 seconds. In systems with a freezing point near 0° C., the period of constant temperature was usually longer. Many of the erratic results were found to follow a behavior that was abnormal in that there was a sudden rise in temperature from the low point during undercooling, followed by an immediate fall with no measurable time interval at the so-called observed freezing point. Such results invariably gave an abnormally low freezing point. This suggested that improper dough packing with consequent air bubbles or incorrect centering of the thermometer, resulted in an appreciable transfer of heat between the thermometer and the salt solution. This local heat transfer might cause the mercury in the bulb to begin contracting before sufficient heat had been released by ice crystallization to raise the system to its true freezing point. In order to check for such irregularities, the time required for the mercury to rebound to the observed freezing point and the time of constancy at this point were recorded with a stop-watch throughout the latter part of the investigation.

A lens was not used as an aid in reading the thermometer, since the observed freezing points could be read to the nearest 0.005° C. with the naked eye. Undercooling was only recorded to the nearest 0.01° C., and in cases when the mercury dropped below the thermometer scale, to the nearest 0.05° C. Since the correction for undercooling involves a fraction of $\frac{1}{80}$, it is evident that an error of even 0.05° C. in amount of undercooling would only appear in the corrected freezing point as an error of about 0.0006° C.

Moisture determinations were made on the flours used, by the official vacuum oven method, at the start of the investigation. They

were found to be low enough in moisture content to preclude any rapid changes in this respect upon exposure to the air of the laboratory during weighing processes. They were stored in tin cans with well-fitted covers to obviate any danger of extensive moisture changes during the investigation.

Several mixers were used for dough preparation in the preliminary studies but the Hobart-Swanson was selected for the major part of the investigation because of its uniform though vigorous action. In this mixer there is little opportunity for any part of the dough to lie undisturbed at any time in the mixing operation.

Doughs were prepared containing only flour and water in a fixed ratio to give a normal consistency. Additional doughs were mixed in which sucrose was an ingredient in amounts sufficient to give 0.125, 0.250 and 0.500 molal solutions of sucrose when using the values obtained for the moisture content of the flour and the water added during dough preparation as the basis for the total potential solvent in the dough. Throughout most of the investigation the precaution was taken of only using sucrose ground fine enough to pass a 60 mesh screen. The required amount of this sugar was stirred into the total water required for dough preparation 20 minutes before use in the dough. It is doubtful if these precautions were necessary to insure complete solvation when dealing with such dilute solutions of sucrose in pure water.

Values for bound water could then be calculated by comparing the freezing points of doughs without sugar with those obtained on doughs containing the three sugar levels as indicated. The method used by Newton and Gortner (1922) and many later investigators was followed in making such calculations. The formula used in such calculations when determining the effect of a 0.5 molal sucrose concentration in a dough is as follows:

$$\% \text{ bound water} = \frac{\Delta_o - \Delta_c}{\Delta_o} \times 94.6,$$

in which Δ_o = the observed difference in freezing point between doughs containing no added sugar and those containing added sugar sufficient to give a 0.5 molal concentration.

Δ_c = the calculated value for the freezing point depression due to 0.5 molal sucrose, assuming a hexahydrate formation (= 0.983° C.).

The calculations for the amount of bound water as indicated by a comparison of the doughs with no added sugar and those with 0.250 molal and 0.125 molal concentrations added, were made in exactly the

same way except that the value of 94.6, representing the percentage of the total water not used in sucrose hydrate formation, was replaced by 97.3 and 98.65, respectively. The calculated values for Δ_c as used for these systems were 0.478° C. for the effect of 0.250 molal sucrose and 0.236° C. due to 0.125 molal sucrose, again based on the theory of hexahydrate formation.

The criticism that this method of calculation does not allow for the increased concentration of the original solutes in the system, due to sucrose addition and hydration, has been mentioned. There is some disagreement as to whether or not this criticism is justified. The correct method of calculation may depend on the particular solutes in the original system. With the concentrations involved in this investigation, the magnitude of the results obtained are little different when using the two methods of calculation as indicated in later discussion.

Materials Used

Three flours were used in this investigation. All were milled from wheat of the 1933 crop but differed widely in strength, as that term is usually defined. Two were milled from different mixtures of hard red spring and hard red winter wheat. One (No. 15790) was a flour of moderate strength intended for household use. The second hard wheat flour (No. 15963) was milled from a mix of higher protein content and was sold as a strong flour for bakers. The third flour (No. 15964) used had been milled from soft wheats and was especially suited for use in making cakes. Differences in these flours, as indicated by the behavior during mixing of doughs containing them, are shown in the plasticity curves reproduced in Figure 2. The composition of these flours with respect to ash, protein, and moisture at the time of use is given in Table I. In the fifth column of this table is also given the amount of water (percentage of flour weight) used in preparing doughs for the freezing point determinations. Doughs prepared with these amounts of added water were judged to be of about equal consistency from each of the flours.

TABLE I

Protein, Ash, and Moisture Contents of Flours and Absorptions Used in Preparing Doughs

	(Crude protein, N × 5.7)	Ash	Moisture	Absorption
	%	%	%	%
Flour No. 15790	11.2	0.42	11.5	64.5
" " 15693	12.3	0.43	11.5	67.0
" " 15694	8.0	0.34	12.2	58.0

The two dry skim-milks described in the preceding paper were also included in one series of doughs prepared for freezing point determinations.

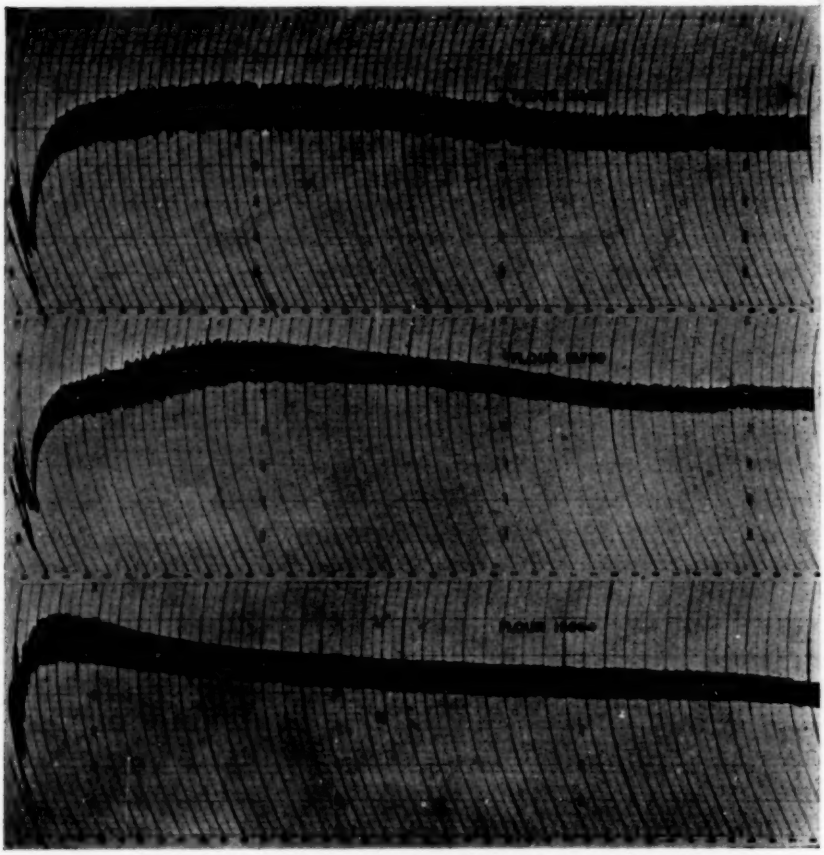

Fig. 2. The effect of three different flours upon the mobility of doughs during mixing as recorded by the Farinograph.

Results

Preliminary determinations of freezing points were made on doughs that had received various mixing treatments. The freezing point of the dough was invariably lowered by mixing but the amount of this depression was very irregular. It soon became apparent that most of the inconsistency in the results was not due to differences in dough properties but was caused by errors in the actual freezing point determinations. The extremely low freezing points often obtained on doughs that had been badly broken by excessive mixing were found to be due to improper centering of the Haidenhain thermometer.

With improving technic it became apparent that mixing exerted a definite effect on the freezing point of doughs prepared with only flour and water or with flour, water and added sucrose. A series of preliminary determinations were made on flour-water doughs therefore, to determine the magnitude of this effect. Duplicate doughs were mixed for each of 1, 2, 3, and 4 minute periods on a Hobart-Swanson mixer, using a stop-watch to time the mixing operation. Three freezing point readings were made on each dough.

The averages of the six freezing point readings made on doughs accorded each mixing treatment indicated a progressive decrease in freezing point with increased mechanical treatment of the dough. The average freezing points after 1, 2, 3, and 4 minutes of mixing were, respectively, —0.527° C., —0.558° C., —0.640° C., and —0.667° C. Many replications made in later comparisons with improved technic exhibited a similar effect.

Dough temperatures were not recorded and no attempt had been made at temperature control in the above series except to use flour and water at about 20° to 22° C. Under such conditions, dough temperatures would invariably be increased by prolonged mixing. It was thought possible that this increase in temperature might be an important factor in the lowered freezing point apparently caused by excessive mixing and that temperature variations might be the cause of some of the variability between replicated doughs.

Accurate temperature control was not possible in the Hobart-Swanson mixing bowl. In a series of comparisons different dough temperature levels were attained by warming or chilling the measured water to be used in the dough and by a slight chilling of the heavy mixing bowl before use in some cases. Doughs containing only flour and water were mixed for two minutes and replicated freezing point readings were taken on each dough. Inconsistencies in results were evident but the general trend is indicated by the data in Table II.

TABLE II

FREEZING POINT OF LIKE DOUGHS AT EIGHT TEMPERATURE LEVELS

	° C.	° C.	° C.	° C.	° C.	° C.	° C.	° C.
Temperature of dough at end of mixing	22	24.5	27	28	30	31.5	35	38
Average of freezing point determinations	−0.525	−0.531	−0.539	−0.547	−0.591	−0.626	−0.660	−0.634

Some effect of temperature during mixing upon the freezing point

of the dough is clearly evident from the data of Table II. Since the temperature range artificially provided in this series is much greater than any that might be caused by heat generation during variable mixing time studies it seemed evident that the cause of the decreased freezing point with increased mixing of the dough could not be entirely due to the temperature variability that had occurred in previous tests.

Some confirmation of the effect of temperature during a ten-minute mixing upon the freezing point of dough was obtained in a later series with another flour. One dough with a temperature of 25° at the end of the mixing operation had a freezing point of —*0.586*° C. Duplicate doughs with a temperature of about 28° C. gave an average value of —*0.600*° C. and another pair at about 32° C. showed a mean freezing point of —*0.670*° C. These freezing points are lower than the values of Table II, due chiefly to the longer mixing time, as will be shown later. However, the magnitude of the temperature effect is nearly the same in the two series.

Some temperature control seemed essential in attempting to determine correctly the effect of mixing upon freezing points and water binding in doughs. By a proper chilling or warming of the water to be used in dough preparation it was usually possible to obtain with reasonable accuracy a temperature of 28° C. in the dough at the end of the mixing period. A lower temperature standard might have been desirable due to less effect of temperature in this range, but it seemed impractical to attempt a lower level due to the prevailing moderate room temperatures and the large amount of heat generated by long treatments in the Hobart-Swanson mixer. When a chilling of the water was not sufficient to keep dough temperatures down to a maximum of 28° during ten-minute mixing periods on certain warm days recourse was had to chilling of the bowl during a part of the mixing process. It is recognized that a constant temperature during mixing would be a better procedure than the one adopted of mixing to a constant maximum temperature. However, the validity of comparisons made after the same mixing treatment is not impaired by the procedure used. Furthermore, the effect of mixing upon freezing points is reduced rather than exaggerated by this method since the mean temperature during a mixing operation would be lower in the doughs mixed for a long period than in those mixed for a short time.

When doughs are prepared in a Hobart-Swanson mixer, definite evidence of physical dough deterioration or breakdown may appear after a 2 to 4 minute mixing period depending upon the ingredients used. The three flours selected for these studies differed widely in the property imparted to the doughs of resisting this deterioration.

TABLE III

THE FREEZING POINTS OF DOUGHS AFTER DIFFERENT MIXING TREATMENTS—*Given as — ° C.*

Dough mixed	1 min.	2 mins.	3 mins.	4 mins.	10 mins.
			Flour No. 15790		
No added sucrose	0.445	0.459	0.475	0.511	0.590
0.125 molal sucrose added	0.936	0.951	0.960	0.959	1.056
0.250 " " "	1.452	1.469	1.480	1.467	1.550
0.500 " " "	2.540	2.585	2.608	2.716	2.714
			Flour No. 15963		
No added sucrose	0.454	0.488	0.527	0.563	0.600
0.125 molal sucrose added	0.904	1.007	1.039	1.073	1.132
0.250 " " "	1.457	1.505	1.479	1.505	1.651
0.500 " " "	2.502	2.588	2.699	2.668	2.814
			Flour No. 15964		
No added sucrose	0.398	0.403	0.401	0.410	0.466
0.125 molal sucrose added	0.893	0.893	0.907	0.940	0.981
0.250 " " "	1.390	1.401	1.404	1.475	1.578
0.500 " " "	2.550	2.602	2.631	2.720	2.816

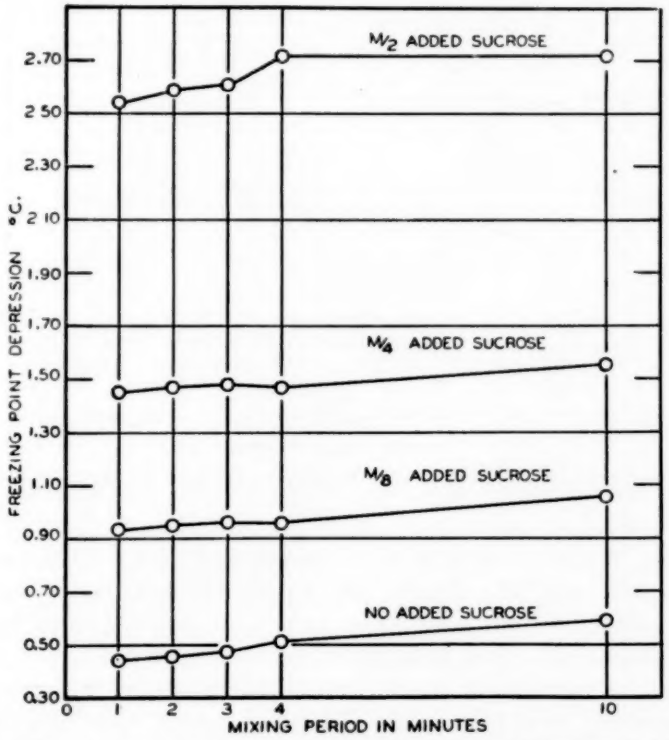

Fig. 3. Freezing points of doughs made with flour No. 15790 and different levels of added sucrose after mixing periods of 1, 2, 3, 4, and 10 minutes. ("M" refers to molality of added sucrose)

To obtain a complete picture of the relationship of this slackening to the water binding in doughs from these flours it was at first considered sufficient to make determinations on doughs after 1, 2, 3, and 4 minutes of mixing. Because of the decided effect that increased mixing appeared to have upon freezing points, the series was extended to include doughs mixed for 10 minutes.

It was deemed advisable to use as low a concentration of added sugar as possible without unduly increasing the percentage error in the deter-

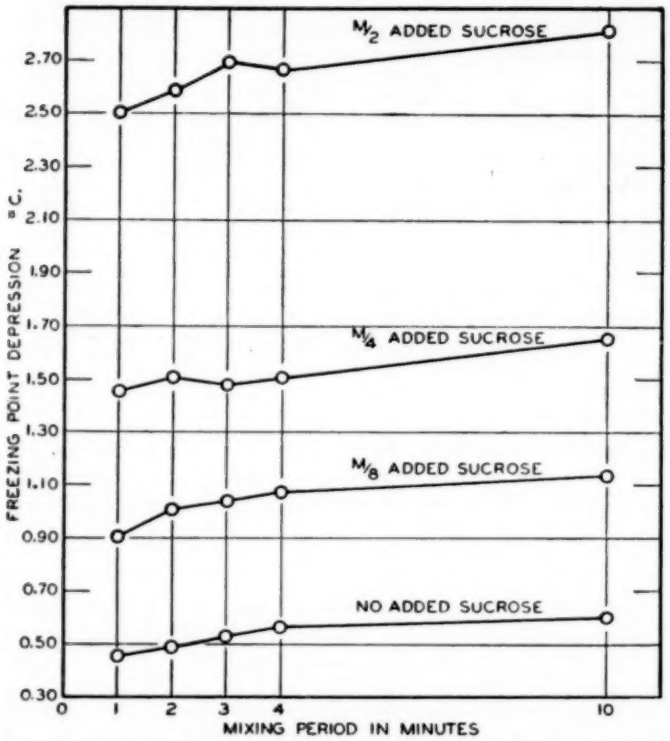

Fig. 4. Freezing points of doughs made with flour No. 15963 and different levels of added sucrose after mixing periods of 1, 2, 3, 4, and 10 minutes. ("M" refers to molality of added sucrose)

mination. To serve as a check on the physical effect of sucrose upon dough quality and also to avoid relying entirely upon the bound water values calculated from a very low sugar concentration, three levels of added sucrose were used throughout the investigation.

After repeating some of the earlier determinations a regularity of effect was observed as a result of mixing doughs containing each of the three flours with water alone and with sucrose in 0.125, 0.250, and 0.500 molal concentration. The averages of the triplicate readings as

obtained on duplicate doughs for each dough type and method of preparation have been corrected for zero point and undercooling and are given as corrected freezing points in Table III.

The obtained freezing-point readings revealed some variability among replicate determinations in many cases which might justify a lack of confidence in the data obtained on any one dough type if it were not supported by other evidence. Such evidence is at hand in the regularity of successive values obtained on the same dough type after increasingly severe mixing treatments, in the similarity of the data from doughs containing different sugar concentrations and by the close agreement shown in the behavior of the three flours used. These trends are more evident in Figures 3, 4, and 5 in which the average

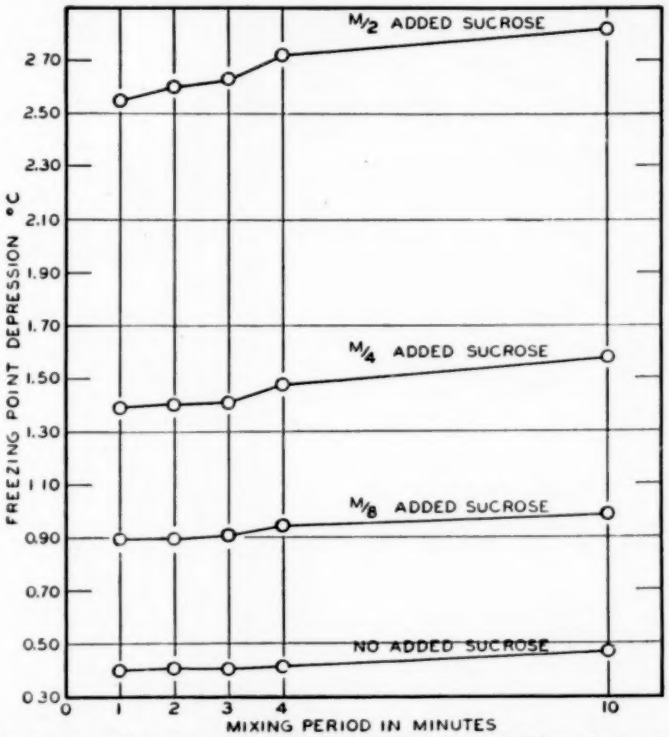

Fig. 5. Freezing points of doughs made with flour No. 15964 and different levels of added sucrose after mixing periods of 1, 2, 3, 4, and 10 minutes. ("M" refers to molality of added sucrose)

freezing points as given in Table III are plotted against time of mixing treatment.

The method of Newton and Gortner, as previously outlined, was used for calculating bound water values from these data. These values as obtained from a comparison of the average freezing points of flour-

water doughs with the freezing points of doughs containing the added sucrose at each of three levels are given in Table IV together with averaged bound water values for each flour, for each mixing treatment, and for each sugar level.

TABLE IV

CALCULATED BOUND WATER VALUES OF DOUGHS AFTER DIFFERENT MIXING TREATMENTS—*As percent of total water*

	1 min.	2 mins.	3 mins.	4 mins.	10 mins.	Av. 5 mixing periods
			Flour No. 15790			
0.125 molal sugar doughs	51.2	51.3	50.6	46.7	48.7	49.7
0.250 " " "	51.1	51.3	51.0	48.7	48.9	50.2
0.500 " " "	50.2	50.9	50.8	52.4	50.8	51.0
Average—3 sugar levels	50.8	51.2	50.8	49.3	49.5	50.3
			Flour No. 15963			
0.125 molal sugar doughs	46.9	53.8	53.2	53.0	54.9	52.4
0.250 " " "	50.9	51.6	48.4	47.9	53.1	50.4
0.500 " " "	49.2	50.3	51.8	50.4	52.6	50.9
Average—3 sugar levels	49.0	51.9	51.1	50.4	53.5	51.2
			Flour No. 15964			
0.125 molal sugar doughs	51.6	51.1	52.6	54.7	53.4	52.7
0.250 " " "	50.4	50.7	50.9	53.6	55.5	52.2
0.500 " " "	51.4	52.3	52.9	54.3	55.0	53.2
Average—3 sugar levels	51.1	51.4	52.1	54.2	54.6	52.7
Grand average—3 flours each at 3 sugar levels	50.3	51.5	51.3	51.3	52.5	51.4

Grand average—3 flours each after 5 mixing periods
0.125 molal sugar = 51.6 0.250 molal sugar = 50.9 0.500 molal sugar = 51.7

Another series of freezing point determinations was made on doughs containing flour No. 15790, water, and 6% dry skim-milk No. 3123, without added sucrose and with sucrose as a dough ingredient to give

TABLE V

THE FREEZING POINTS OF DOUGHS CONTAINING FLOUR NO. 15790 AND DRY SKIM-MILK AFTER DIFFERENT MIXING TREATMENTS—*Given as* —° *C.*

Mixed	1 min.	2 mins.	3 mins.	4 mins.	10 mins.
6% dry skim-milk No. 3123—No added sucrose	0.967	1.018	1.071	1.107	1.141
6% dry skim-milk No. 3123—0.250 molal sucrose added	2.032	2.025	2.073	2.155	2.280
6% dry skim-milk No. 3124—No added sucrose	0.979	0.975	1.076	1.068	1.242
6% dry skim-milk No. 3124—0.250 molal sucrose added	1.997	2.020	2.075	2.086	2.262

a 0.250 molal concentration when based on total potential solvent. Doughs were mixed for 1, 2, 3, 4, and 10 minutes as in the previous series. The series was duplicated using dry skim-milk No. 3124 instead of No. 3123 due to the formers decidedly different effect on dough quality. A water absorption of 68%, based on flour weight, was allowed in preparing all doughs supplemented with these dry milks. Of the total of 20 doughs prepared with these two milks, with and without added sucrose, with each of five mixing treatments, duplicates were only obtained for 12 due to the exhaustion of this flour supply. The average corrected freezing points as obtained from triplicate readings on single or duplicated doughs are given in Table V.

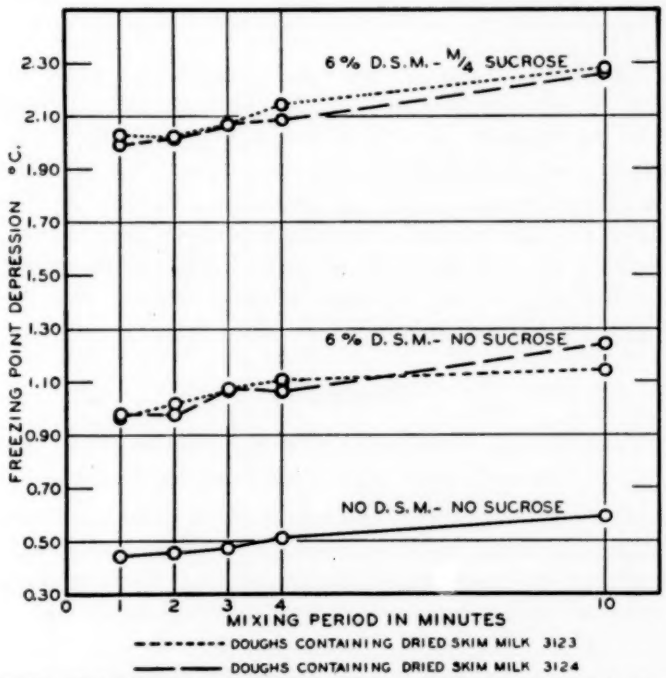

Fig. 6. The effect of two different dry skim-milks upon the freezing points of doughs made with flour No. 15790. ("M" refers to molality of added sucrose)

The data of Table V is also graphically presented in Figure 6 in which freezing points are plotted against time of mixing treatment. The curve showing the freezing points of this flour alone, as given in Figure 3 is also again reproduced in this graph to show the effect of the dry skim-milks upon the freezing points of the doughs.

Bound water values in the doughs containing the dry skim-milks have been calculated from the data of Table V. Since only one level of added sucrose was used in this series, only one set of bound water

values are available being that obtained by a comparison of the freezing points of doughs without added sugar and with sucrose added to give a calculated 0.250 molal concentration. These bound water values calculated in the same manner as previously used are given in Table VI.

TABLE VI

CALCULATED BOUND WATER VALUES IN DOUGHS CONTAINING DRY SKIM-MILK AFTER DIFFERENT MIXING TREATMENTS—*As per cent of total water*

Mixed	1 min.	2 mins.	3 mins.	4 mins.	10 mins.	Average
Doughs with D.S.M. No. 3123	53.6	51.1	50.9	52.9	56.5	53.0
Doughs with D.S.M. No. 3124	51.6	52.8	50.7	51.6	51.7	51.7

There is little evidence of difference in the bound water values in the doughs containing the two milks in spite of the pronounced difference in the physical characteristics of these doughs. The slight irregularities and particularly the higher values after 10 minutes of mixing with the doughs containing dry skim-milk No. 3123 may be entirely due to experimental error which is more pronounced due to less replication than in the previous series. The supplementary milk solids apparently have a higher water binding capacity than the flours studied, as will be shown in subsequent discussion.

Discussion

1. THE VALIDITY OF METHODS USED FOR DETERMINING FREEZING POINTS AND FOR CALCULATING BOUND WATER

Reference has already been made to the suggestion of Newton and Martin that when using cryoscopic technic on plant saps, it was necessary to replicate the freezing point determinations on fresh samples due to an apparent change in protein hydration by freezing and thawing. No evidence of any such change has appeared in these studies of flour doughs. This is most conclusively proven by averaged values which have been obtained for the successive freezing point readings. From 335 doughs, the average 1st reading was $-1.428°$ C., 2d reading $-1.426°$ C. and 3d reading $-1.431°$ C. This proves that no error involving changes in protein hydration was introduced by successive freezing and thawing with the adopted technic since no trend of increasing or decreasing values is evident. Doughs might be expected to be less sensitive in this particular than a system such as a plant sap and especially so in this case since excessive ice crystal formation was avoided as much as possible.

Two assumptions made in using the correction for undercooling may be subject to error. One assumes that the specific heat of the system is that of water and this value has been used since the specific heat of dough is not accurately known. Any error would be practically constant in the systems investigated, would not be of large magnitude, and would only slightly affect the computed bound water values since it would be almost equally introduced in doughs both with and without added sugar. The latter statement is only true if undercooling is of the same magnitude in both dough types. Averaged values for the undercooling obtained in this investigation are as follows:

Doughs with no added sugar	3.41° C.
" " M/8 " "	3.58° C.
" " M/4 " "	3.54° C.
" " M/2 " "	2.71° C.

It is evident that the undercooling obtained at the two lower sugar levels at least, was nearly identical with that obtained in doughs with no added sugar so that this assumption does not affect the validity of the results.

The second assumption is that no heat transfer occurs from the freezing mixture to the ice bath during the period of sufficient ice crystallization to reach the maximum temperature or observed freezing point. It has been noted that erratic results were invariably those with an excessively low freezing point. It is believed that such results have usually not been caused by ordinary heat losses from the dough but by a direct transfer of heat from the thermometer bulb to the ice bath. This direct local transfer of heat might be possible because of air bubbles in the dough layer or improper centering of the thermometer resulting in only a thin film of dough between the thermometer bulb and the salt solution. This salt solution would be approximately at the lowest temperature attained in undercooling. Too direct a contact between the thermometer bulb and this solution could cause the mercury to fail to expand as much as it should in order to accurately record the temperature rise occurring in the dough during the period of sufficient heat release by ice crystal formation to reach the observed freezing point. The possibilities of such errors were reduced to a minimum in the latter part of the investigation. The error introduced by the normal transfer of heat from dough to ice bath during the temperature rebound after undercooling would be constant on all doughs since a nearly constant amount of undercooling and time required for such rebound was found to hold in this investigation. It should not be a factor in the calculated bound water values.

Since erratic readings were invariably in the direction of excessively

low freezing points, it might be assumed that the freezing points showing least depression would be most nearly correct. Calculations were made to determine the magnitude of the obtained bound water values if only the highest (least depressed) reading obtained on each dough was used. Freezing point values thus selected were also plotted in the same manner as the averaged data of Figures 3, 4, and 5. The resultant curves were no more regular than those obtained when using the average of all freezing points. The bound water values thus calculated also showed about the same regularity and magnitude as those previously tabulated. There was usually a slight reduction in bound water values by using this method of selection due to a greater average spread in freezing point values obtained on doughs with added sugar than in those without added sugar. The magnitude of this correction ranged from an increase of 0.2% to a decrease of 3.0% in the case of extreme doughs. The reduction in bound water values when averaging the results from all doughs was 1.1% when using highest freezing points for each dough rather than average values as the basis for calculation. This different method of using the data does not give significantly altered values.

The contention made by Grollman has already been mentioned in which he maintains that the method used for calculating bound water is in error because it does not correct for the concentration of original solute caused by sucrose hydration. Grollman proposes the following formula:

$$\text{Percentage bound water} = \frac{\Delta_a - \left(\frac{1000}{892}\Delta + Km\right)}{\Delta_a - \frac{1000}{892}\Delta} \times 89.2.$$

Δ_a = Freezing point of system containing added sucrose in molal concentration.

Δ = Freezing point of system without added sucrose.

This formula has been applied in the calculation of bound water values for 10 of the doughs containing added sucrose selected at random from the freezing point data already presented in Table III. The necessary correction of constants in Grollman's formula was made to make it applicable to the 0.125, 0.250, and 0.500 molal sugar concentrations included in this series. It was found that bound water values were lowered by 0.4% to 0.8% when making this correction for original solute concentration. The average reduction in calculated bound water for the ten doughs was 0.6% below the values recorded in Table IV. It is evident that no discussion of the merits of Grollman's criticism is

necessary in connection with the presentation of these data since the reduction caused by the use of his formula is of no significance in the systems under consideration.

2. The Significance of the Calculated Bound Water Values

The percentage of the total water present that appears to be bound in bread doughs as determined by this technic, is among the highest values that have been reported. The percentage of total moisture present in bread doughs is lower than that in most other systems that have been investigated from this standpoint. Thus, even with the high bound water percentages, the amount of water bound per unit weight of dry matter is not as high in such systems as many that have been previously reported.

Reference has already been made to the work of Newton and Cook on the bound water of flour suspensions. Because of the technic used in their investigations, limitations were placed upon the total solids concentrations that could be used. As a result of relatively low concentrations of flour in their suspensions, low values were obtained for bound water calculated as percentage of total water. Because of the low bound water values and the relatively large errors involved in the freezing point technic, they deemed it impractical to make extensive determinations on natural flour-water suspensions. Acidulation was resorted to thus increasing hydration capacity. Washing out of electrolytes was also practiced in several of the reported tests to again effect an increase in hydration capacity. Such practices were necessary in order that the observed differences in freezing point might be great enough to have some significance. Because of these treatments of the systems used, prior to the determination of freezing points, a direct comparison cannot be made of the values obtained by them on flour-water suspensions with those now reported on flour-water doughs.

When attempting to compare systems that differ widely in solids to water ratios, it may be more valid to consider the percentage hydration or amount of water held as bound per unit weight of the solid material, rather than the percentage of total water that appears to be bound. In the investigations of Newton and Cook it was found that when using untreated flour-in-water suspensions with a 15% flour concentration that the percentages of water apparently bound "were about 2%." This indicates a hydration of the flour of about 11% of its own weight when considering hydration to be the taking up of the water that is recorded as bound by this technic. When acidulating to a pH of 3.0, unwashed commercial flour-in-water suspensions gave a hydration value as high as 50% when using a flour concentration of 4.5% but this value

was progressively lowered by increasing concentration until at 18.1% flour concentration a hydration value of only 36% was indicated.

Hydration percentages have been calculated for the averaged bound water values as given in Table IV. Due to the different absorptions allowed these flours for dough preparation, as listed in Table I, the averaged hydration values for these flours are not in the same order as the results for bound water. The lowest hydration value is shown by the cake flour (No. 15964) with 42.1%. The medium strength bread flour (No. 15790) is intermediate with 43.2%, while the strongest flour (No. 15963) gave a hydration of 45.4%. All of these values are higher than those reported by Newton and Cook for the highest concentrations studied by them even when acidulating the flour-water suspension. Probably the physical alterations in protein structure incident to the formation of dough results in increased water holding capacity.

In Newton and Cook's experiments a hydration of about 30% was indicated for starch from wheat flour. By difference, it was determined that the hydration of the proteins in acidulated flour-water suspensions was about 100% in unwashed and 200% in washed suspensions. The hydration of glutenin and gliadin prepared from these flours by Osborne's method averaged only about 85% in acidulated suspension, thus showing a possible reduction in hydration capacity due to the treatment necessary for purification.

Hydration capacities of the three flours in this series are in the order of protein content. If a 30% hydration is assumed for all the non-nitrogenous solid material in these flours, values for the hydration of the proteins are obtained as indicated in the last column of Table VII.

TABLE VII

CALCULATED HYDRATION OF FLOUR PROTEINS BASED ON AN ASSUMED HYDRATION OF 30% FOR NON-PROTEIN SOLIDS

Flour Number	Total moisture	Crude protein	Non-protein solids	Total hydration	Fraction of hydrated water held by non-protein solids	Fraction of hydrated water held by proteins	Hydration of proteins in flour
	%	%	%	%	%	%	%
15964	12.2	8.0	79.8	42.1	23.9	18.2	227
15790	11.5	11.2	77.3	43.2	23.2	20.0	179
15963	11.5	12.3	76.2	45.4	22.9	22.5	183

Although it is recognized that other components besides starch are present in the non-protein solids of flour, they exist in small amounts.

Since the 30% hydration value as obtained for starch was only an approximation, any correction for other non-nitrogenous constituents in flour would probably not add to the accuracy of the calculations.

The differences in the estimated hydration capacities of the proteins in these flours may be of no significance. The average value is nearly twice that obtained by Newton and Cook for proteins in unwashed flour-water suspension even when acidulating to increase hydration capacity. This acidulation was shown to have a marked effect on the amounts of bound water in flour-water suspensions although having only a small effect on starch prepared from these wheat flours. An increased hydration of flour, due to dough formation probably principally due to changes in protein structure or aggregation, is again indicated by a consideration of the data from this viewpoint. The effect of various concentrations of acid and other ions upon the bound water values of bread doughs would be of considerable interest in this connection.

The effect of about 2% of common salt upon the water imbibing capacity of doughs would be especially interesting since this is the level usually used in bread production. It is evident that the addition of such a substance in systems studied by cryoscopic technic would introduce complexities in the matter of calculating and interpreting results. The systems selected for this initial study of the freezing points of doughs were made as simple as possible to avoid such complexities. Furthermore, studies with the Farinograph have revealed that the effect of such salt levels upon the water imbibing capacity of doughs is not very pronounced. The addition of this amount of salt usually causes a slightly higher mobility when passing through the minimum during the mixing of a dough as compared to the level attained without the use of salt. Stability of the dough is somewhat increased by the salt addition. Thus, it is not likely that the imbibition or water-binding capacity of a dough will be greatly altered by the addition of such amounts of salt as are usually used in bread production. These observations as to the small effect of a salt only apply to doughs with normal acidity and electrolyte content and would probably not apply if dealing with acidulated or electrolyte-free flour preparations.

The hydration capacity of the milk solids may be calculated from the freezing points obtained with milk-free doughs and with doughs containing 6% dry skim-milk, each compared with the freezing points of these systems containing sucrose in 0.250 molal concentration. Only this sugar level was used in the milk doughs and the tests were only conducted with flour No. 15790. The amount of water bound per 100 g. moisture-free flour as shown by the milk-free doughs containing no added sugar and those containing 0.250 molal sucrose on averaging

doughs accorded all mixing treatments was 43.1 g., or 38.1 g. for each 88.5 g. of moisture-free flour. In the milk doughs, 6 g. of dry skim-milk was used for each 88.5 g. of this moisture-free flour. When the bound water percentages of all milk doughs are averaged and consideration is given the moisture content of milk and flour and the water absorptions used, it may be calculated that 88.5 g. of moisture-free flour and 6 g. dry skim-milk apparently held 41.7 g. bound water. It accordingly appears that 6 g. dry skim-milk or about 5.8 g. of moisture-free milk solids held (as bound water) 3.6 g. of water. This is a hydration capacity of 62.1% as compared to 43.1% for the flour solids in the same system.

This high hydration capacity of dry skim-milk might be expected in view of its high protein content of about 37%. If it is assumed that all the water apparently bound by the milk solids is held by the protein components, then a hydration capacity by the milk proteins of 168% is indicated. The proof of water binding by milk solids when present in bread doughs may not be complete due to the fact that considerable variability was noted in the percentages of bound water in milk doughs that had received excessive mixing and these rather high values are included in the averages given above. Howat and Wright (1934) have recently reported an inability to find evidence of water binding in milk solids-water systems. The results of the present investigation may indicate a different capacity for water binding when milk solids are present in bread doughs than when in water alone.

3. The Significance of the Observed Changes in Freezing Point Caused by the Extended Mixing of Doughs

Although bound water values are apparently not significantly affected by extended mixing of doughs, the decided lowering in freezing point invariably caused by such treatment is of considerable interest. The freezing point data, as given in Table III, reveal that the freezing point of doughs was appreciably lower in those doughs mixed for 10 minutes than in those having received a 1 minute mixing treatment. When the data for the three flours were averaged, this lowering of freezing point was found to be 0.120°, 0.1450°, 0.1930° and 0.251° C. for doughs with no added sugar and with added sucrose in 0.125, 0.250, and 0.500 molal concentration, respectively. This lowering of freezing point was of such magnitude in the various doughs containing sucrose in differing concentration that the calculated bound water values showed no significant change due to mixing. Thus there is no change indicated in the amount of available solvent. But the lowering of freezing point in all doughs gives evidence of an appreciable increase in water-soluble materials due to mixing.

The most direct measure of this increase in solute can be obtained on doughs with no added sugar. The two bread flours gave almost identical decreases in freezing point when calculating the difference in average values for the freezing points of doughs with no added sucrose that had been mixed for 1 and 10 minutes, respectively. These decreases were 0.145° and 0.146° C. For the cake flour in the series the corresponding value was 0.068° C.

Such decided effects on the freezing points indicate an appreciable increase in solute. This additional solute might owe its origin to the release of normally water-soluble materials that had been more or less firmly held by the dough complex in the early stages of mixing or to the formation of new water-soluble compounds from insoluble materials during the mixing operation. In the former classification could be placed sugars and salts adsorbed by, or chemically combined with the proteins (and possibly the starches) of the system.

Many investigators have found it impossible to entirely wash flour suspensions free of electrolytes. The gradual removal of soluble material as obtained on repeated extractions with water indicates an original occlusion or binding of these substances. It is not unreasonable to believe that some of the salts and sugars normal to wheat flour would be chemically or physically included in the gluten network when first formed during the mixing process. The severe treatment accorded doughs by prolonged mixing might so alter the configuration of the proteins in this network that linkages would be broken in this depolymerizing or rearranging process thus releasing soluble ions.

Possibilities in the formation of solute from insoluble materials would include principally the change of starch into sugars or soluble dextrins and the formation of soluble nitrogenous compounds from the insoluble proteins in the system. It has been believed that such changes occur so slowly in normal bread doughs that only an insignificant amount of such soluble materials could appear in a 10 minute mixing period. Some of the wheat flour proteins are normally water-soluble but these should have little effect on freezing points due to their high molecular weights and should have the same effect in doughs mixed for 1 minute as in doughs mixed for 10 minutes unless some of these compounds are linked into protein complexes in such a way as to be unavailable until released by severe mechanical treatment. In the previous paper Skovholt and Bailey (1935) there was shown no appreciable increase in non-precipitable (so-called non-protein) nitrogen as a result of severe mixing of doughs. This may not preclude the possibility that some decrease in size of protein aggregation has occurred in sufficient amount to have some effect on the freezing point of the system.

Of the above-mentioned possible sources of increased solute only the change of starch to reducing sugars can be simply and accurately measured. A few tests were made on the flours used in this investigation to determine the amount of reducing sugar apparently formed during the mixing of doughs.

4. The Effect of the Reducing Sugars Formed During Mixing Upon the Observed Freezing Point Changes

In 1931, Skovholt and Bailey presented an unpublished report to the American Dry Milk Institute in which were cited certain experiments on the diastatic activity of flour suspensions and doughs. In these studies it was found that the apparent reducing sugar level in bread doughs had been tremendously increased in a brief mixing period over the values obtained on freshly prepared flour-water suspensions. Recently a series of investigations has been conducted by Steller, Markley, and Bailey (1935) in which the apparent rate of reducing sugar formation in flour-water suspensions has been determined. Digestion periods of 15 minutes to 2 hours were allowed in these studies of 19 flours. These results show that over 70% of the total reducing sugar produced in a 1 hour period had been formed in the first 15 minutes. About 65% of the total found after a 2 hour digestion was present after the first 15 minute interval.

No report is known of results that have been obtained when digestion periods of less than 15 minutes have been used. Nearly all studies of sugar production from the starch of flour have been made on suspensions due to difficulties encountered in preparing doughs for a sugar determination. No attempt will be made to review the literature on diastatic activity since the determinations of this type were only incidental to the main purpose of this investigation.

Doughs were prepared with only flour and water using a variable period of mixing in the Hobart-Swanson mixer. An aliquot of dough equivalent to 20 g. of flour was immediately cut into small pieces and dropped into a tared wide-mouthed bottle containing about 30 cc. water, 10 cc. of 10% H_2SO_4, and 10 cc. of 12% sodium tungstate. This mixture was agitated for exactly 1 minute with a high speed "malted milk" stirrer. By this time the mixture in the bottle had been reduced to a smooth suspension. It was then made up to 200 g., centrifuged, filtered, and an aliquot of the filtrate taken for a determination of reducing sugars by the method proposed by Blish and Sandstedt (1933) as an adaptation of Hagedorn and Jensons (1923) method for the estimation of blood sugar.

Determinations were made on doughs prepared from each of the

three flours used in preceding tests, each after mixing for 1 minute and for 10 minutes. With one of the flours several other intervals of mixing treatment were used ranging upward from 15 seconds. Dough ingredient temperatures were adjusted to give a dough from the mixer at near 28° C. in all cases. After a few preliminary determinations it was found that a fairly constant time of about 2 minutes was required to remove a dough aliquot from the mixer, weigh it and place it piece by piece into the water-acid-tungstate mixture.

Determinations of the amount of reducing sugars present in the flours were also made on undigested suspensions. These values were obtained after the treatment accorded the doughs including the one minute agitation with the stirrer. The only difference was in the elimination of the preliminary treatment in the mixer for dough preparation. Average results from these studies are given in Table VIII in which the values are expressed as milligrams of maltose per 10 g. of flour.

TABLE VIII

REDUCING SUGARS IN DOUGHS AFTER DIFFERENT DURATIONS OF MIXING TREATMENT

Results as milligrams of maltose per 10 g. of flour

Duration of mixing	15 secs.	30 secs.	45 secs.	1 min.	4 mins.	10 mins.	Flour suspension blank
Flour No. 15790				106		136	18
Flour No. 15693	96	100	101	107	141	143	18
Flour No. 15694				78		89	14

Values were obtained for reducing sugars present in buffered suspensions of these flours after a 1 hour digestion period. This method as proposed by Blish and Sandstedt gave values of 227, 214, and 94 mgs. of maltose per 10 g. of each of these flours, respectively, in the order of listing in Table VIII. Unbuffered suspensions would give somewhat lower results than the above.

The results emphasize the extreme rapidity of increase in reducing sugars during the first moments after the addition of water, even though the time intervals as given in the table cannot be taken at face value as the total period allowed for digestion, due to the delay of about 2 minutes between the end of the mixing period and enzyme inactivation. The high values after such brief digestion periods might cause speculation as to whether or not some of the increase in reducing sugars may not be caused by chemical changes or the breaking of linkages other than that normally attributed to enzyme action. The data ob-

tained is neither extensive enough nor was the experiment properly planned to give definite information on this point.

The parts of the data in Table VIII of principal interest in this investigation are the values for reducing sugars obtained after a 1 minute and a 10 minute mixing of doughs. Increases of 30, 36, and 11 mgs. of maltose per 10 g. of flour are indicated by this extension of mixing time on doughs containing the three flours. By referring to Table III it will be noted that the average decrease in freezing point due to this additional 9 minute mixing of doughs containing no added sugar was 0.145°, 0.146°, and 0.068°, respectively, listing results with the different flours in the same order as above. It may be noted that the cake flour which showed the least effect of mixing upon freezing point is apparently low in enzymatic activity as judged by the production of reducing sugars.

The time interval between the end of mixing and enzyme inactivation was probably about the same in the technics for determination of freezing points and for reducing sugars. Accordingly the effect of the increased amount of reducing sugars upon the freezing points can be approximately calculated. The determined increases in reducing sugars expressed as milligrams of maltose per 10 g. of each of the flours were converted into terms of mols of soluble material. By taking into account the moisture contents of the flours and the amount of water added in dough preparation the calculated depressions of freezing point due to these increased amounts of sugars were determined. For the bread flours the average value of this calculated depression was .023° C., and for the cake flour it was found to be about .009° C. Thus the determined increase in reducing sugars accounted for about 16% of the observed freezing point depression in the former case and about 13% in the latter.

This hydrolysis of starch to reducing sugars would probably be accompanied by an increase in the amount of intermediate products. Some of these intermediate products might be sufficiently water soluble and present in great enough concentration to have an appreciable effect upon the freezing point of the system. A similar hydrolysis of proteins has been mentioned as a possibility although difficult of proof. Reactions of this type in addition to increasing the soluble material of the system also require water, thus further increasing the effect on the freezing point. This water requirement would exert a relatively small effect as compared to the increase in solute concentration.

The increase in solute concentration in doughs due to excessive mixing is not of large magnitude as indicated by freezing points. It may be sufficient, however, to be associated with the plasticity decreases and

the increases in stickiness observed in varying amounts in different bread doughs. An interesting point in this connection was the almost complete absence of stickiness during any stage of the breakdown of the doughs containing the cake flour used in this series. A decided increase in soluble materials is probably not the cause of increased stickiness but may be a result of changes in aggregation and a breaking of linkages that is in some way responsible for this common characteristic of overmixed doughs.

The appearance of overmixed doughs suggests that the water is less firmly held after such severe mechanical treatment. The results of this investigation indicate that no appreciable change has been made in the percentage of the total water which is held with sufficient firmness to be unavailable as a solvent under the conditions of these experiments. The partial breakdown of some of the colloidal components or micelles might reduce the amount of water held by co-valent linkages but might free certain polar groups from their association with each other and thus enable them to serve as nuclei for the binding of water. Such an attack upon the protein network of a dough could result in an increased mobility without causing any change in the percentage of water unavailable for solvent action. The freeing of soluble fractions and possibly of adsorbed ions by the rearrangements caused by drastic mechanical treatment could cause a change in the electrical charges on the surface of the colloidal components of sufficient magnitude to give rise to the condition known as stickiness or adhesiveness. Such speculations are unsupported, however, except by the observed parallelism of increased solute formation with changes in physical dough characteristics.

Summary

A technic is described for use in obtaining freezing points directly in slightly mobile systems such as bread doughs in attempting to determine if different degrees of water-binding capacity exist in doughs that differ in physical properties due to a variable mechanical treatment.

Variable temperatures in doughs as a result of a variable mixing time was found to be a factor in the obtained freezing points making it necessary to bring all doughs from the mixer at the same temperature.

Replicated determinations were made of the freezing points of doughs with each of three flours, each in turn at four sugar levels after mixing treatments of 1, 2, 3, 4, and 10 minutes. Calculated bound water values were not significantly different as a result of variable mixing and were approximately the same in doughs containing each of the three flours.

The average bound water value obtained as a result of these determinations was 51.4% of the total water present. When allowance was made for the variable amount of water used in dough preparation, it was found that the percentages of bound water held by the solids of the different flours were slightly different and in the order of protein content. These hydration capacities ranged from 42.1% to 45.4% when calculating bound water held per unit weight of dry matter.

Two dry skim-milks of different baking quality were similar in their effect upon the percentage of water bound in doughs after variable mixing treatment. An average hydration capacity of 62.1% was shown by moisture-free milk solids.

Significantly lowered freezing points of all doughs resulted from an increase in the mixing time due apparently to increasing concentrations of solute. Extended mixing apparently caused increases in amount of reducing sugars sufficient to account for 13% to 16% of the observed decrease in freezing point when comparing doughs mixed for 1 and for 10 minutes. Other possible sources of the increased amount of solute are suggested. There is postulated a possible relationship of this solute formation to observed changes in dough plasticity during mixing and to increases in adhesiveness.

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A STUDY OF THE AVAILABILITY OF BREAD CARBOHYDRATES [1]

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In a cooperative study of the nutritional values of bread which has been carried on in several laboratories during the past year our part was to determine and compare the availability of the carbohydrates of white, wheat, and rye breads.

Accurate determination of the nutritional value of carbohydrates in foods and food materials is complicated by major difficulties which do not apply or are far less important in studies of protein or mineral utilization. These difficulties have been well stated by Morgan, Strauch, and Blume (1930), who point out that none of the several methods which have been employed to test the biological availability of carbohydrates is adequately standardized or consistently reliable.

The most extensive studies of the nutritive value of breads were conducted by Rubner (cited by Lusk, 1928) during the World War under compulsion of the semi-famine conditions then prevailing in Germany. Rubner employed the conventional method of determining digestibility by comparing food and fecal analyses. Conclusions based upon this method, particularly with respect to carbohydrate assimilation, must take into account the uncertain extent of bacterial decomposition in the intestine and, at best, do not extend to the fate of such materials as may be absorbed.

It would appear that the most acceptable method of determining the digestibility and absorbability of carbohydrates is based upon use of phlorhizinized animals. Among studies which have employed this method of assay may be cited those of Olmsted (1920) upon carbohydrates of certain vegetables, and Adolph and Kao (1934) upon soybean carbohydrate.

Inasmuch as availability of a foodstuff to the organism is dependent not only upon the degree to which it may be digested and absorbed, but also upon the extent to which the absorbed products of digestion may be metabolized, we have undertaken to determine the first of these

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two conditions for bread carbohydrates by assays upon phlorhizinized dogs, and the second condition by observations of the reducing materials excreted in the urine of bread fed normal dogs.

The breads which we have thus tested were obtained from two Memphis bakeries which have a wide and representative distribution in the local and surrounding territory.

The first problem in technique was to prepare bread in sufficiently large lots to assure representative samples, and in such condition that it might be kept for considerable periods of time without deterioration. This problem was solved by the construction of a special drier which holds at one time 6 short loaves of sliced bread. It will be noted from Figure 1 that individual slices of bread in the trays of the drier are

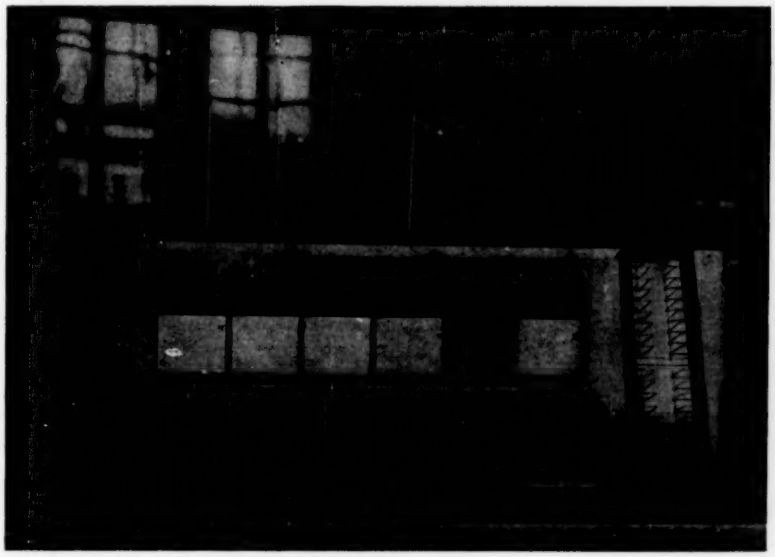

Fig. 1. Bread drier.

separated so that the heated air may pass between them. Air, driven by two small electric fans across electrically heated coils, passes from the top air-duct through small circular openings and emerges through duplicate openings into the bottom air-duct. Air volume and the number and diameter of openings in the air-ducts were so calculated that a fall in temperature of only 3 to 4° occurs between the first and last compartments.

After 2½ hours in the drier at a temperature of 100 to 105° the initial moisture content of 30 to 35% is reduced to less than 2%, with no toasting and only a slight yellowing. The bread was then finely

ground, thoroughly mixed, and kept in stoppered glass bottles. In this state the bread will keep apparently more or less indefinitely. For certain experiments the crust portion of the dried slices was scraped off, and the crust and inside portions ground separately.

TABLE I

In Vitro ANALYSES OF BREADS

Type	Lot number	Moisture		Nitrogen		Carbohydrate as glucose: Acid hydrolysis[3]		Carbohydrate as glucose: Taka-diastase[4]	
		Fresh[1]	Dried[2]	Fresh	Dried	Fresh	Dried	Fresh	Dried
		%	%	%	%	%	%	%	%
White bread	W-1	33.5	0.5	1.52	2.28	53.3	80.3	51.9	78.1
" "	K-1	26.5	3.2	1.71	2.42	58.9	80.2		
" "	K-2	34.8				53.3	81.8		
" "	K-3	33.4				55.7	83.7	53.7	80.6
" "	K-4	33.4	0.8	1.64	2.46	56.7	85.2	52.2	79.0
" " (Crust)	K-2		1.6		2.42		81.8		
" " (Inside)	K-2		1.8		2.44		82.8		
Whole wheat bread	K-1	35.6	1.7	1.71	2.66	50.9	79.1	45.8	71.2
" " "	W-1	34.2	1.3	1.53	2.43	50.3	76.3	47.8	72.7
Rye bread	K-1	37.1	1.0	1.78	2.83	50.0	79.5	45.8	73.0
" "	W-1	32.1	1.2	1.47	2.16	55.0	81.0	52.2	76.8
Arrowroot starch							97.8		95.5

[1] Weight loss of fresh bread after drying 2½ hrs. in special drier at 100–105°.
[2] Further loss in weight of dried bread after grinding and heating 1 hr. at 100–110°.
[3] Mixture of 2 g. sample, 200 ml. H_2O, 20 ml. 8.6 N HCl, refluxed 2½ hrs., over free flame.
[4] Mixture of 2 g. sample, 200 ml. H_2O, 100 mgm. Taka-diastase, incubated 15 hrs. at 40°. Then 20 ml. 8.6 N HCl added and digested 2½ hrs. on water bath.

In Table I are summarized the moisture, nitrogen, and carbohydrate values of the several lots of bread used in our experiments. Attention is called to the consistently lower values obtained for carbohydrate (as glucose) when the initial hydrolysis was accomplished by Taka-diastase followed by mild acid hydrolysis on the water bath (Olmsted, 1920) as compared with acid hydrolysis over the free flame. This difference may be attributable to cellulose converted by the more drastic hydrolysis. Accordingly, we have taken the Taka-diastase values for comparison with the *in vivo* values obtained upon the phlorhizinized dog.

Altogether, 17 bread feeding experiments and, for control, 5 starch feeding experiments have been carried out upon phlorhizinized dogs. Female dogs, ranging in weight between 12 and 18 kilos, were employed. Urine periods were marked off by catheterizing. A typical individual experiment, from which may be noted the general plan and conduct of such experiments, is shown in Table II.

In calculating the extra sugar, originating from the carbohydrates of the bread and excreted in the urine, the assumptions are made that, since fasting phlorhizinized dogs do not pass feces even after ingesting so small a quantity of bread, all of the nitrogen of the bread is excreted in the urine, and the corresponding bread proteins are glucogenic in the

same ratio to nitrogen as the prevailing endogenous D: N ratio of the fore and after control periods.

TABLE II

Dog 2. Initial weight, 12.95 kg.; final weight 11.35 kg. Fasted 40 hrs. prior to first injection of phlorhizin, Dec. 17, 9.00 a.m. Thereafter, daily injections of phlorhizin at 9.10 a.m.

Date	Time of collection	Urine vol.	Acetone	Reaction litmus	Total N per hour	Total sugar per hour	D : N	Calculation
1933		*Ml.*			*Grams*	*Grams*		
Dec. 19	9.00 a.m. to							
	1.00 p.m.	52	tr.	acid	0.41	1.69	4.15	
	5.00 "	66	++	"	0.45	1.67	3.71	
" 20	9.00 a.m.	220	++++	"	0.40	1.36	3.39	
	11.00 "	28	+++	"	0.42	1.52	3.62	
			Total for 26 hours		10.65	38.16	3.58	
At 11.05 a.m. dog was fed 25 g. white bread (Lot K-1) equivalent to 20.04 g. glucose								
Dec. 20	2.00 p.m.	111	+++	acid	0.43	3.62	8.43	Average control D : N = 3.49
" "	5.00 "	106	tr.	"	0.37	2.94	7.86	Endogenous sugar = 7.58 × 3.49 = 26.42 g.
" 21	9.00 a.m.	270	++++	"	0.29	1.48	5.17	Extra sugar = 19.75 g.
	11.00 "	24	++++	"	0.28	1.37	4.85	Recovered 98.5%
			Total for 24 hours		7.58	46.17	6.08	
Dec. 21	2.00 p.m.	48	++++	acid	0.31	1.10	3.59	
	5.00 "	38	++++	"	0.33	1.17	3.53	
" 22	9.00 a.m.	173	++++	"	0.34	1.14	3.35	
	11.00 "	20	+++	"	0.35	1.15	3.31	
			Total for 24 hours		8.06	27.35	3.40	

All of the carbohydrate assays upon phlorhizinized dogs are summarized in Table III. It will be noted that average recoveries in the urine of glucose equivalents of the fed breads are largest for wheat and smallest for rye. Individual variations are considerable but, we believe, not larger than are to be anticipated in this type of experiment. After long experience in the behavior of phlorhizinized dogs we are convinced that a quantitatively successful assay of the glucogenic value of a fed material is largely determined by the degree of depletion of glycogen stores in the tissues. If the glycogen has been too far depleted, some glucose from the fed material will be retained as glycogen beyond the period allowed for its excretion; if tissue glycogen has not been depleted to the optimum, equilibrium level, extra sugar will still be coming from this source. The exact optimum period for the feeding cannot be predicted with certainty or estimated from the immediately preceding urine analyses, since the latter cannot be completed rapidly enough. Hence, selection of the feeding time is based largely upon known average behavior.

Another important consideration which would account in part for

individual variations in the phlorhizin assays is the delayed and prolonged absorption of carbohydrate incident to digestion of the bread fed. It will be recalled that phlorhizin diabetes is an extreme renal diabetes, the drug so lowering the renal threshold for glucose that a profound

TABLE III

SUMMARY OF ASSAYS IN THE PHLORHIZIN DOG OF THE CARBOHYDRATE VALUES OF BREADS

Dog	Bread fed		Glucose equivalent of bread fed as determined by taka-diastase hydrolysis	Per cent of glucose value of bread found as extra sugar in urine of phlorhizinized dogs
	Type	Lot number / Amount		
		grams	*grams*	%
2	White bread	K-1 25		102.5
11	" "	K-4 25	19.8	78.5
14	" "	K-4 25	19.8	88.5
9	" "	W-1 15	11.7	80.0
8	" "	K-3 25	21.1	73.4*
			Average	87.4
15	Whole wheat bread	K-1 25	17.8	92.3
10	" " "	K-1 15	10.7	116.0
3	" " "	K-1 25	17.8	114.0
12	" " "	W-1 25	18.2	102.0
10	" " "	K-1 25	17.8	78.7*
			Average	106.1
8	Rye bread	K-1 25	18.3	85.3
7	" "	K-1 25	18.3	70.3
5	" "	K-1 25	18.3	93.2
4	" "	K-1 25	18.3	77.5
13	" "	W-1 25	19.2	78.8
5	" "	K-1 25	18.3	85.5*
7	" "	K-1 25	18.3	59.0*
			Average	81.0
16	Arrowroot starch	20	19.1	70.5
17	" "	20	19.1	95.5
18	" "	20	19.1	85.3
13	" "	20	19.1	82.2*
15	" "	20	19.1	66.0*
			Average	83.8

* Not included in average; result of the second of two feeding periods in a single phlorhizin regime

hypoglycemia is established. At the low level of blood sugar the pancreas is not normally stimulated to secrete insulin [2]; the condition is an

[2] It is generally accepted that insulin supplied by the pancreas is responsive to blood sugar concentration (*cf.*, for example, Kosaka (1933)). Other mechanisms to account for variations in carbohydrate tolerance have been proposed, particularly by Himsworth (1934) and by Soskin, Allweis, and Cohn (1934).

exaggerated hunger diabetes. When 20 to 30 g. of pure glucose are fed in a single dose the blood sugar is temporarily elevated, even considerably above normal, and the pancreas is stimulated to increased rate of insulin secretion. However, there is so considerable a lag in this pancreatic response that the fed glucose has been absorbed and excreted before sufficient insulin is available to effect the metabolism of some part of it. Hence, in such a case the recovery in the urine of fed glucose is reasonably quantitative. If, however, the first glucose feeding be followed in a few hours by a second feeding the glucose now absorbed meets insulin secreted in response to the first stimulus, and a considerable fraction of the second feeding may be metabolized. Thus it is that in the later stages of digestion of bread the absorbed sugar may be in part utilized. Likewise, the lower results shown in Table III after a second bread feeding in the same phlorhizin period are explicable in terms of the considerations here presented.

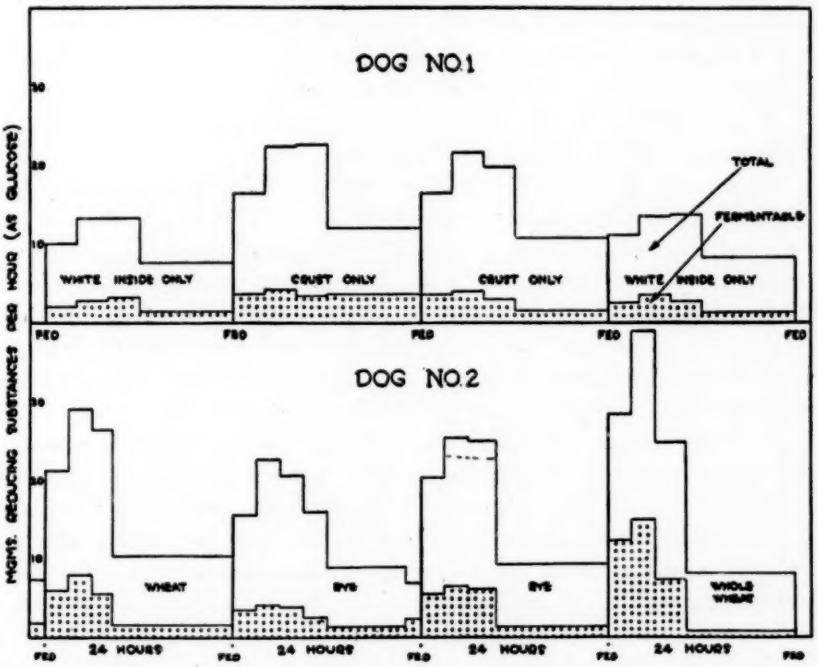

Fig. 2. The excretion of total and fermentable reducing substances in the urine of the normal dog on diets of various kinds of bread.

Dog No. 1. Fed daily 250 g. dried Kroger Country Club bread (wheat bread). This is equivalent to 370 g. fresh bread, or one small loaf. Showing the effect of feeding the white inside and crust parts of the bread.

Dog No. 2. Fed daily 250 g. dried Winkleman "Taystee" bread. This is equivalent to 370 g. fresh bread, or one small loaf. Showing the effect of feeding wheat, rye, and whole wheat.

It would seem that selected experiments which disclose apparently optimal conditions and yield maximal recoveries in carbohydrate assays

upon phlorhizinized animals, rather than average data of an entire series of experiments, are more valid for such conclusions as may be drawn.

When starch was fed (Table III), in the form of a cooked thick paste, recoveries as extra glucose in the urine ranged from 70 to 95%, with an average of 83.8%. Inasmuch as this average is only slightly above that for the lowest of the breads, whereas individual experiments may be selected for each type of bread in which recovery through the animal was better than 90% of the *in vitro* carbohydrate value, we are disposed to conclude that essentially the total non-cellulose carbohydrate content of bread is made available to metabolism in so far as digestion and absorption are concerned. The indication in our experiments that the carbohydrates of rye bread may be somewhat less completely assimilated would, we believe, require a larger series of experiments to confirm it. The higher *in vivo* determined values for whole wheat bread we believe are attributable to the unexplained more rapid digestion and absorption of the carbohydrates in this type of bread, a relationship which is illustrated in Figure 2.

In Figure 2 we have a comparison not only of the rates of excretion of sugar in the urine of phlorhizinized dogs after feeding the three types of bread, but also in the rates of excretion of reducing substances in the urine of normal dogs upon a bread diet.

Three normal dogs, 15 to 17 kilos initial weight, were maintained in perfect condition and with small weight increases for periods of 1, 2, and 4 months, respectively, upon exclusively bread diets. Each dog received daily at one feeding 250 g. of the dried and powdered bread mixed with enough water to make a thick dough. White bread was fed except for short intervals when rye or wheat bread was substituted. In none of the experiments was there any indication that the animals could not have been maintained indefinitely upon the bread diet.

Urinary excretion by these dogs of total and fermentable reducing substances [3] was followed during successive short intervals of numerous 24 hour periods. The analytical methods here used were those developed by West and Peterson (1932), involving a preliminary removal of nitrogenous reducing substances by precipitation with acid mercuric sulphate and barium carbonate, and measuring the reduction in filtrates, before and after yeast fermentation, by the Shaffer-Hartmann technique.

Aside from the significant fact that mammals whose metabolism is so closely akin to the human may be maintained in an apparently perfect nutritional state for prolonged periods upon a diet of bread alone, these experiments upon normal dogs demonstrate an insignificant wastage in metabolism of carbohydrates supplied by bread feeding. Even if we

[3] We expect to report at a later date certain observations upon the chemical character of reducing substances found in the urine of dogs fed bread diets.

assume that the total reducing materials in the urine of these dogs represent waste carbohydrate, the total excretion of 200 to 400 mgs. per 24 hours is but 0.1 to 0.2% of the daily intake of approximately 200 g. of bread carbohydrate.

While probably of no importance in considerations of practical nutrition, it is of interest to note in Figure 2 that the urinary excretion of reducing substances, both total and fermentable, is largest upon a diet of wheat bread and lowest for rye bread.

In Figure 3 are comparisons of the urinary excretion of reducing substances when the diet was 250 g. per day of crust and crust-free

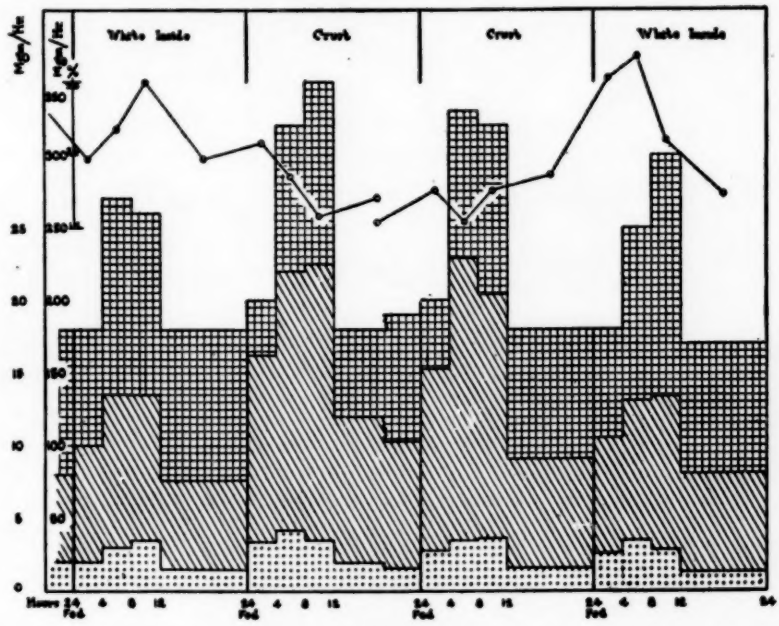

Fig. 3. Excretion of total nitrogen and total and fermentable reducing materials in the urine of a normal dog after feeding crust and crust-free portions, respectively, of white bread. Four consecutive 24-hour periods are represented. At the beginning of each period 250 g. of the dried bread (see text) were fed. Excretion is still at a maximum in the 4-hour period 8 hours after feeding. After ingestion of crust there is a considerable increase in nitrogen and total reducing substances above the corresponding values when the inside portion of bread was fed. No comparable increase occurs in the fermentable fraction of the reducing materials. In the figure the dotted areas represent fermentable reducing substances, the cross hatched areas represent non-fermentable reducing substances, and the squares represent total nitrogen. Per cent of total reducing material as fermentable is shown in the circled line graph.

portions, respectively, of white bread. Upon the crust diet the total excretion of reducing substances was approximately 50% higher than for the inside portion of the bread, with little change in the fermentable fraction. When crust was eliminated from the bread diet the animals suffered a mild degree of constipation which was corrected promptly by feeding crust alone or returning to the whole bread diet.

Conclusions

From assays upon phlorhizinized dogs, and observations of the reducing substances excreted in the urine of normal dogs after bread feeding, it is concluded that essentially the whole of the non-cellulose carbohydrates of white, wheat, and rye breads are available for nutrition.

Evidence is offered that the carbohydrates of wheat bread are absorbed more rapidly than are the carbohydrates of white or rye bread.

There is some indication that the carbohydrates of rye bread are assimilated less completely than are the carbohydrates of white or wheat breads.

Dogs have been maintained in excellent condition upon exclusively bread diets for periods up to 4 months, with no indication that they could not have been continued indefinitely in good nutritional state upon a diet of bread alone.

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THE BIOLOGICAL VALUE OF THE PROTEINS OF WHITE, WHEAT, AND RYE BREADS[1]

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In the past the measurement of the nutritive value of bread has been the object of considerable research. Since changes arise in the quality and kind of raw materials used, as well as in the process of manufacture, the question as to how these changes affect the nutritive value of bread is a perennial one.

The work given in this paper has for its purpose the nutritive evaluation of the proteins of ordinary white, whole wheat, and rye breads. Bread "as purchased" from a large local bakery was fed in this project and may be considered as representative of the product consumed by the public. In order to ensure the widest applicability of results, human subjects were used in feeding trials, as well as white rats.

In the past many investigators have used either growth or nitrogen balance studies as criteria for the worth of the dietary protein. Hindhede (1914) claimed that the proteins of bread, meat, and potatoes can replace those of the body gram for gram, but his work has been criticized because of the assumption in his comparison that fecal nitrogen was wholly of food origin. Abderhalden, Ewald, Fodor, and Röse (1915), like Hindhede, concluded that bread nitrogen is as good as potato nitrogen and that for both the minimum nitrogen for maintenance was somewhat lower than Hindhede's value. Rubner (1917), in extensive experiments with variously modified white and rye breads, obtained nitrogen equilibrium at an intake of 10 to 11 g. of nitrogen. Neumann (1919) kept himself in nitrogen equilibrium over a period of 7 months with a daily intake of 7 g. of nitrogen from rye bread, but he also used a very high calorie intake (73). Martin and Robison (1922) attempted a repetition of these experiments with a lower calorie intake (45 to 50) and did not attain equilibrium until 10.8 g. of nitrogen from rye bread was fed. These and other results are confusing, rather indecisive and difficult of comparison.

[1] Presented before the Biological Section at the 88th meeting of the American Chemical Society at Cleveland, Ohio, September 10–14, 1934.

Thomas (1909), who conceived the idea of biological value of proteins, fed himself a nitrogen-free diet to obtain the minimal loss of body protein and compared the relative sparing action of many food proteins to this one base: The biological value being the number of parts of body protein spared by 100 parts of the food protein. This conception of the biological values of proteins proved to be very stimulating. Mitchell (1923), in this country, perfected the technique for use with rats, and many workers in different laboratories have secured fairly consistent results on a wide variety of proteins. Because of these considerations Mitchell's procedure has been followed as a standard in the development of this work, which will be presented more fully elsewhere.

Experimental

Tests with Rats

30 female rats at three stages in their life-cycle were fed a protein-free diet, and diets in which the protein at about a 5% level was furnished by the protein of either milk, or white, whole wheat (50–50), or rye (25–75) breads. The feeding periods lasted ten days, the first four being preliminary, in which the food intake was adjusted to the level where the rat would just clean up all its food. Complete nitrogen balances upon each animal were made for each period.

The data obtained on the two-thirds grown animals are not given because of difficulties encountered in producing a minimum nitrogen excretion. Even after ten days on a nitrogen-free diet the rats were still eliminating considerable quantities of nitrogen which came from previous feeding. These data are mentioned only to indicate one of the difficulties of experimentation, and will form a section of a future report.

TABLE I

Average Biological Values

From other laboratories			In this study		
Food protein	Level fed	Biological value	Bread protein at 5% level	Age of rat	Biological value
Whole wheat	6	68[a]	White	Young	74
Whole milk	7	93[b]	Whole wheat	Young	73
Eggs	8	93[c]	Rye	Young	66
Pork	8	74[c]	White	Mature	83
Heart	8	74[d]	Whole wheat	Mature	80
Liver	8	77[d]	Rye	Mature	82

[a] Boas-Fixsen, M., and Jackson, H. M., Biochem. J. **26**: 1923 (1932).
[b] Mitchell, H. H., J. Biol. Chem. **58**: 905 (1924).
[c] Mitchell, H. H., and Carman, G. G., Ibid. **60**: 613 (1924).
[d] Mitchell, H. H., and Beadles, J. R., Ibid. **71**: 429 (1926–27).

The figures for the biological values of the breads as obtained with young and mature rats appear in Table I. The definitely lower biological value obtained with younger animals for each of the breads indicates that they require more bread protein for the replacement of an equivalent quantity of body protein than the older animals. The rapidly growing animal needs protein for both maintenance and growth; the requirements for these two functions are not the same and are practically inseparable; the lower biological value in young animals therefore suggests that the broader requirement of a growing animal should be less well covered than that of an older animal by anything other than a perfect protein. This suggestion is also supported by the fact that the protein maintenance requirement of the animal appears to decrease with age. It follows also that the requirements of the growing animal are more critical. An example of this is seen in the slightly inferior utilization of rye bread by the growing animals. The white and whole wheat breads were milk breads; the rye was not. Osborne and Mendel (1919) and Sherman and Campbell (1924) have shown that milk proteins have a supplementing action upon the proteins of grains. The lack of milk in the composition of the rye bread appears to correlate with its lower biological value.

For purposes of comparison the biological values of a few proteins are also given in Table I. It appears that, except possibly for young animals, bread proteins may be classed with meats and are inferior only to milk and eggs.

Tests with Human Subjects

The program of the experiments on human subjects was carried out as follows: In the first and last periods milk furnished the dietary protein, white bread in the second, rye bread in the third, and whole wheat bread in the fourth. The experimental periods lasted six days except for the first which was seven, three of which were preliminary.

An example of the diets as consumed may be seen in the record of the food intake of one of the subjects of the experiment, in Table II. The diets were prepared so as to furnish to each of the six subjects 0.1 g. nitrogen and 50 calories per kilogram of body weight and only slight variations from this standard were necessary during the course of the experiments.

Following somewhat the plan of Murlin [2] in recent cereal studies, 70% of the nitrogen in the diet came from bread, 20% from cream, and 10% from the fruit and other foods.

Cream, butter, jam, fruit, and beverages were given in the same daily quantities to each person during the experiment. This departure

[2] Murlin, J. R., Personal communication.

TABLE II

RECORD OF FOOD INTAKE

(Period 1, Subject 3.)

Food	Intake		Nitrogen		Calories	
	Per meal *Grams*	Daily *Grams*	% [a]	*Grams*	*Per gram* [b]	*Total*
Butter	20	60	.073	.044	7.94	476
Jam	30	90	.023	.021	3.41	306
Orange	135	135	.122	.165		
Banana	75	75	.135	.101		200
Prunes	45	45	.316	.143		
Coffee	150	300	.037	.110		
Cocoa	4 (dry)	4	3.880	.155		
Sugar	20	60			4.00	240
Cream	100	300	.403	1.209	2.00	600
Milk	263	790	.552	4.361	.715	564
Starch bread [c]	83	249	.043	.107	3.70	914
Totals				6.416		3300
Per kilogram body weight				0.10		52

[a] As analysed.
[b] Atwater, W. O., and Bryant, A. P., Bull. 28, rev., U.S.D.A. (1906).
[c] Prepared by a formula recently developed in Dr. Murlin's laboratory at the University of Rochester, and kindly placed at our disposal.

from a more rigorously limited diet was made in the belief that the more nearly feeding conditions approximated the normal the closer the real nutritive value of bread in the ordinary dietary would be approached. As has been pointed out by several investigators in nutrition, any determination of the nutritive value of a single foodstuff may have little practical worth because of the supplementing action of one foodstuff on another and the fact that a single food is seldom consumed by itself. Furthermore, the difficulties with bowel irregularity that oftentimes invalidate short-time experiments on the more restricted diets, especially with human subjects, have not been a problem.

Twenty-four hour collections of urine were made throughout the experiment, and were analyzed immediately. For the last three days of each period, four in the case of the first, feces were collected and kept frozen until analysis could be made upon the composite samples. Nitrogen analyses were checked in duplicate upon the urines, in triplicate on the food and feces.

The data for complete nitrogen input and output obtained on one subject are exhibited in Figure 1. The food N (2) [3] is practically constant, and for this subject the fecal N (4) likewise, although none of the subjects showed much variation in this particular. The daily urine

[3] Figures in parenthesis refer to the lines in Figure 1.

N (3) varies, often directly with the volume output of the urine (1). Control of the volume of the urine was quite impossible because of the extremely hot weather. The change from one bread to another did not influence significantly the height of N excretion in the urine, but the change to milk lowered it markedly.

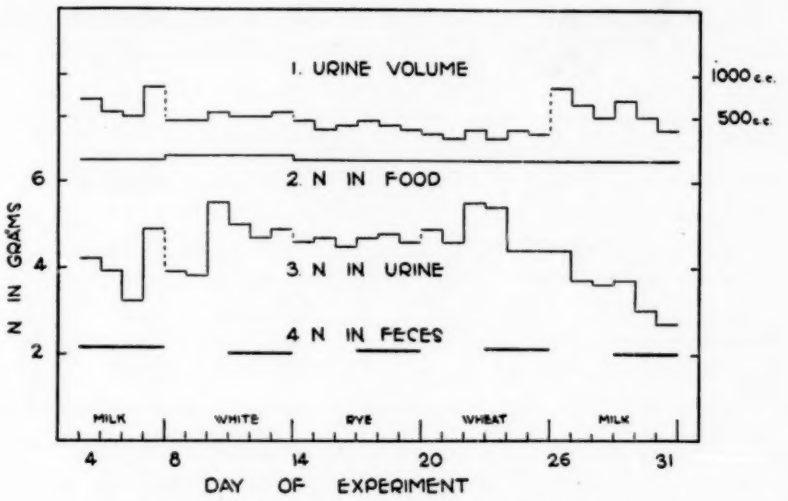

Fig. 1. Intake and excretion of Subject 2 for entire experiment.

All subjects remarked on the laxative effect of whole wheat bread. With this bread the rate of passage of the fecal markers through the alimentary tract was speeded up about three times. The duration of the journey changed from 24 to 36 hours to only 6 to 12 hours. The number of satisfactory defecations was increased also.

In order to obtain numerical values for the experimental results a procedure was followed similar to that used in the ordinary computation of biological values, as follows:

$$1.\ \text{B.V.} = 100 \times \frac{\text{intake Np}-(\text{fecal Np}-\text{fecal Npf})-(\text{urinary Np}-\text{urinary Npf})}{\text{intake Np}-(\text{fecal Np}-\text{fecal Npf})}$$

which may be simplified to its equivalent:

$$2.\ \text{B.V.} = 100 \times \frac{\text{Maintenance N} + \text{Balance N}}{\text{Absorbed N}}$$

In these equations Np = the N of the protein-feeding test period and Npf = the N of the protein-free test period.

In formula 1, above, the data of the milk period take the place of data from a nitrogen-free feeding period. It should make no difference which were used if the biological value of milk is 100 (which it approximates in rats), and milk is 100% digestible. In other words, the "maintenance N" in formula 2 may be represented by the N excretion of either a protein-free or maintenance-milk feeding period.

The biological values of the bread proteins as determined in human subjects are given in Table III. The lower value for milk at the beginning than at the end of the month-long experiment indicates that, at the start, a constant level of nitrogen excretion had not been attained. Probably some "circulating protein" held over from previous feeding was excreted in this period. This is evidence additional to that already mentioned that satisfactory data for biological value determination can be obtained only after a sufficiently long feeding period on a low nitrogen intake.

TABLE III

BIOLOGICAL VALUES OF BREAD PROTEIN IN HUMAN SUBJECTS BASED ON 100 FOR MILK IN A FINAL PERIOD

Protein of diet	Subject number						Average
	1	2	3	4	5	6	
Milk	95	86	70	68	85	96	83
White bread	85	74	83	91	89	78	83
Whole wheat bread	82	75	80	88	97	79	83
Rye bread	81	75	84	90	94	77	83
Milk	—	—	—	—	—	—	(100)

The biological values show clearly that there is considerable variability between individuals as to the degree the bread protein is utilized as compared to milk but that there are no significant differences in the values of rye, white, or wheat bread proteins to the same subject. The variability noted between subjects may be due to different individual utilizations of the milk proteins, a suggestion that has the doubtful support of the fact that Subjects 4 and 5 were biased by their previous tastes in their dislike for milk and the possible correlating fact the same subjects gave the highest values to the bread proteins (or vice versa, a lower value to milk).

Attention should also be called to the fact that close agreement was attained between the biological values as determined on mature rats and adult humans even though the basis for evaluation in the first case was body protein, and in the latter milk protein. Perhaps it should be mentioned as confirmatory evidence for this agreement that milk protein gave an average biological value of exactly 100 in the same group

of experimental rats. This evidence is thus in favor of the assumption that biological values as determined on the rat are of the same order as those determined on humans.

Summary and Conclusions

The biological values for maintenance for the proteins of white, whole wheat (50–50) and rye (25–75) breads as determined on young and mature rats and human subjects are given.

The young rats did not utilize the protein of rye bread as well as that of the milk-supplemented white and whole wheat breads, nor could they utilize any of the bread proteins for their maintenance needs as well as could the mature animals.

When determined on adult animals, either human subjects or rats, the biological values of all the breads were of the same order.

Changes in the processes of manufacture have not significantly affected the nutritive value of breads and we are led to conclude as did Hindhede (1914) and Sherman and Campbell (1924) that the proteins of bread may be grouped in the same class as meat proteins and are inferior only to the proteins of the highest biological value such as are contained in milk and eggs.

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HEMOGLOBIN PRODUCTION IN RATS ON DIETS CONTAINING BREAD [1]

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It is now recognized that the value of a food as a source of iron and copper depends on the amounts of these elements present in forms which the body can absorb and utilize. Elvehjem (1932) has shown that inorganic iron compounds can be used by anemic rats for hemoglobin production while organic iron, such as that present in hematin, is not absorbed. The copper of the yolk of egg is not available to the body because of the easily split sulfur compounds that are present; copper sulfide is apparently formed and this is not absorbed (Sherman, Elvehjem, and Hart, 1934). The availability of the copper and iron of bread has not been determined by the newer methods. As far as the total quantities of these essential elements are concerned, bread has been considered as a relatively poor source of iron and only a fair source of copper. Our analyses of different breads, as recorded in Table I, are in harmony with this view.

Whole-wheat bread contains as much as twice the amounts of iron and copper present in white bread. The values for rye bread are intermediate. From a practical standpoint the iron furnished by such quantities of bread as can be readily consumed may supply a significant portion of the daily requirements for this element. An intake of 15 mg. of Fe per day, as suggested by Sherman, is widely accepted as a satisfactory standard. Lintzel (1929), however, has reported experimental evidence which indicates that a normal adult may maintain iron equilibrium on intakes of less than 1 mg. per day. As an indication of the possible significance of bread as a source of iron it may be stated that eight average slices of whole-wheat bread No. I (Table I) would provide about 3 mg. One cannot estimate the importance of bread as a source of copper, because the copper requirements of the body have not yet been defined.

The most satisfactory method for the assay of the available iron and copper in bread consists of feeding anemic rats and analyzing the

[1] Presented before the Biological Section at the 88th meeting of the American Chemical Society at Cleveland, Ohio, September 10–14, 1934.

blood to determine the production of hemoglobin. This was the procedure selected for the present study.

TABLE I

AVERAGE IRON AND COPPER CONTENTS OF BREAD

Variety	Brand number	Moisture	Fe	Cu
		%	*Mg. per 100 g., dry basis*	*Mg. per 100 g., dry basis*
White	I	36.6	1.19	0.26
	II	37.0	1.20	0.28
	III	37.6	1.20	0.30
	IV	35.0	1.02	0.35
Whole Wheat	I	38.0	2.51	0.40
	II	36.4	1.89	0.67
	III	35.5	1.54	0.65
Rye	I	35.4	1.67	0.41
	II	35.9	1.81	0.32

Young rats were made anemic by restriction to a diet of cow's milk, essentially according to the technique of Elvehjem and Kemmerer (1931). The cows were milked directly into glass vessels. The milk contained an average of 0.12 mg. of Cu and 0.18 mg. of Fe per liter. When the rats were extremely anemic they were given daily supplements, to be described, and the response noted by measuring the changes in weight, hemoglobin concentration of the blood and the number of red blood cells. The methods employed have been described by Bing et al. (1934).

To determine the value of bread as a source of copper, the anemic rats were given daily supplements of 0.25 mg. of Fe as pure $FeCl_3$ solution and 4 g. of air-dried bread. In four weeks these animals had doubled in weight, and had attained a normal number of red blood cells and a hemoglobin concentration which was three times the initial level.

Other animals were given, in addition to iron, one of the following supplements: 0.005 mg. of Cu as $CuSO_4$ solution, 5 drops of viosterol, 0.1 g. of glutamic acid. The glutamic acid and the viosterol were tested because there is some evidence that these substances, which would be supplied by the sample of bread tested, may play a part in hemoglobin production. Another group of rats received supplements of iron alone.

The results are summarized in Table II. It may be concluded from these data that, under the conditions of the experiments, iron alone is ineffective for hemoglobin production. Further, the addition of either viosterol or glutamic acid, contrary to what has been claimed by Beard et al. (1933) and Fitz-hugh et al. (1933), had no demonstrable value. All of the rats receiving these supplements died without showing any

marked improvements in weight, number of red blood cells or hemoglobin concentration of the blood. As shown in Table II the average daily intakes of both iron and copper were computed. These data show that the anemic rat requires more than 0.0025 mg. of Cu per day, when the copper is furnished by cow's milk. The addition of copper either in the form of $CuSO_4$, or small amounts of bread, permits growths and hemoglobin production. To determine the quantitative utilization of the copper of bread would require many more experiments. From the data available, however, it is evident that the utilization of bread copper is at least two-thirds, and probably more, as effective as the utilization of inorganic copper.

TABLE II

WHITE BREAD AS A SUPPLEMENT TO IRON FOR HEMOGLOBIN PRODUCTION IN RATS

Supplement [1]	Number of rats	Time	Average weight		Average hemoglobin		Average red blood count		Average intakes per day	
									Fe	Cu
		Days	*Grams*		*Grams*		*Millions*		*Mg.*	*Mg.*
			Beg.	End	Beg.	End	Beg.	End	Per day	Per day
White bread	8	28	80	165	3.2	10.0	4.1	10.0	0.30	0.0130
		120		315		14.7		10.2	0.28	0.0158
Cu	5	28	70	112	3.2	8.3	3.7	9.3	0.25	0.0078
		120		217		12.8		10.6	0.26	0.0108
None	9	26 [2]	69	64	3.4	2.5	4.1	2.7	0.25	0.0020
Viosterol	6	27 [2]	71	61	2.5	4.5	3.6	4.8	0.25	0.0013
Glutamic acid	5	11 [2]	54	53	2.8	2.5	3.1	2.7	0.25	0.0025

[1] Basal diet: Milk + 0.25 mg. Fe per rat per day.
[2] All these rats died and the figures represent average survival periods.

The rats receiving bread grew better than the animals receiving $CuSO_4$. Although the copper intakes of the two groups are not identical they are of the same order of magnitude, so that it is reasonable to conclude that the presence in bread of elements other than copper is responsible for the better growth. The stimulating effects of manganese on the growth of mice fed a milk-iron-copper ration has been reported by Kemmerer, Elvehjem, and Hart (1931).

The iron content of white bread is insufficient to permit the study of its utilization by the methods applicable to iron-rich foods. That the iron of bread, though small, is available to the body was demonstrated by feeding newly weaned rats as much bread as they would eat. In order to balance the diet each rat was given 15 cc. of fresh whole milk each day. On this diet the experimentally determined intakes per day averaged 0.08 mg. of Fe and 0.019 mg. of Cu per rat. Nearly all of the iron and 91% of the copper was derived from the bread. The

intake of copper is satisfactory but the amount of iron is insufficient for optimal hemoglobin production.

However, considering the low intake of iron, the growth of the rats on the bread and milk diet was unusually good. The females grew about as well as litter mates placed on a stock diet. The males also grew well. In 200 days they had grown from 35 g. to over 350 g. but they did not attain the weight of litter mate males reared on a stock diet. The hemoglobin level of the blood of the experimental rats, after 200 days, averaged 12 g. per 100 cc. of blood, as compared with 14 g. per 100 cc. for rats fed on the stock diet. These data show that the iron of white bread can be utilized by the rat for growth and hemoglobin production. The amount of utilization, however, is not shown by these experiments.

Evidence of the extent of utilization was obtained by experiments on the value of whole-wheat bread. Anemic rats were permitted to eat this bread *ad libitum*, and each rat received also 15 cc. of fresh milk daily. Seven rats initially weighed 74 g. and had 3.5 g. of hemoglobin per 100 cc. of blood and 3.6 million red cells per c.mm. After feeding on the bread and milk for 28 days these rats weighed an average of 155 g., and had 12.5 g. of hemoglobin per 100 cc. of blood and 9.1 million red cells per c.mm. The blood was normal for rats of the same weight. The average intake of copper was 0.037 mg. per rat per day and, of iron, 0.22 mg. per rat per day. The performance of these rats equalled that of rats in our colony (for example, the records in Table II) that have received similar amounts of iron in the form of ferric chloride. It is therefore concluded that the iron of whole-wheat bread is in a form that permits its efficient utilization by the anemic rat for growth and the production of hemoglobin.

Determinations of the inorganic iron of whole-wheat bread were made and showed that about 50% of the total iron is in this form. This would indicate that only half of the iron of bread is in a form available to the body. The feeding experiments, however, indicate practically complete utilization. Differences in the intakes of copper may account for this discrepancy. Further experiments will indicate whether the copper content of the diet must be more clearly defined in assays of the utilizable iron of foods or whether in certain cases organic iron is also available.

Summary

Analyses show that some whole-wheat breads contain twice as much iron and copper as white bread. Rye bread contains more copper and iron than white bread but less iron than whole-wheat bread. Feeding experiments show that both the copper and iron of bread can be effi-

ciently utilized by anemic rats for growth and the production of hemoglobin. White bread is a fair source of copper but is a poor source of iron. Whole-wheat bread is not an iron-rich food, although in amounts that might readily be consumed it could supply a significant portion of the daily requirements of normal adults. The iron of whole-wheat bread appears to be equivalent to inorganic iron for hemoglobin production, although only 50% of the total is in this form. It is pointed out that differences in the copper intakes of the experimental animals may explain this discrepancy between biological and chemical assays.

Further study of the chemical method for the estimation of inorganic iron indicates that nearly all of the iron in bread should be considered inorganic.

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OBSERVATIONS ON THE NUTRITIONAL VALUE OF BREAD IN THE DIET OF HUMAN SUBJECTS AND EXPERIMENTAL ANIMALS [1]

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Introduction

During the first three decades of the twentieth century, the per capita consumption of wheat flour (Northwestern Miller Almanac, 1934) in the United States declined by 31%, due chiefly to a decrease in the popular use of bread.

Factors effecting this change in the food habits of the nation not only are of interest to the student of nutrition but also they demand serious study on the part of the baking and allied industries because 60% of all bread used is purchased from bakeries by the consumer.

Some authorities (Sherman, 1933, p. 574) have attributed the diminishing use of bread primarily to a decrease in the demand for energy producing foods, dependent upon the steadily increasing replacement of human labor by machines in the last two decades. This explanation, however, is not supported by the statistics of Pearl (1920) which indicate that during the past twenty years the decrease in the average man's daily intake of calories is not more than 2%.

Notions, popularized by faddists, concerning the alleged nutritional deficiencies or deleterious effects of food products prepared from cereals may have been a secondary cause effecting the reduction in the consumption of bread. For example, largely due to the frequently quoted hypothesis of Green and Mellanby (1928) that cereals may predispose to rickets there is a belief that cereals or foods prepared from cereals may be harmful in the human dietary because they retard the normal processes of calcification. However, Cowgill, Jones, Frisch, and Jackson (1927) observed no signs of rickets in rats maintained on diets of which from 65–93% of the calories were supplied as cereals and these authors (1927a) state that their results demonstrate conclusively that cereals may be used by man to a much greater extent than is common in this part of the world and that nutritive success with high cereal rations is primarily a problem of providing suitable supplements.

[1] Presented before the Biological Section at the 88th meeting of the American Chemical Society at Cleveland, Ohio, September 10–14, 1934.

Another trend in the changing food habits of the nation which may be a secondary factor affecting the consumption of bread is the restriction of bread or its elimination from the diet indiscriminately with other sources of carbohydrate by men and women who subscribe to the unbalanced, reducing diets in a desperate effort to establish or to maintain slender body forms at any price. A fallacy in the indiscriminate restrictions of bread in reducing diets is that consideration is given only to the caloric value of the bread in comparison with other sources of carbohydrate. The facts are ignored that bread is an economic source of proteins of high biological value and that these proteins through their specific dynamic actions affect man's energy metabolism.

Although bread has been an essential part of the human dietary for centuries and man through experience has worked out combinations of bread with other foodstuffs, appealing to the appetite and adequate for his varied needs, there has been reported no comprehensive survey of the nutritional merits of bread as offered to the consumer by the baking industries.

Five laboratories are collaborating in an intensive study of various phases of the nutritional assets of bread, as purchased on the market in four centers of the United States. For the first time the experimental results obtained by the collaborating investigators during the past year will be presented in this symposium.

In this general program, our part included two projects: (a) observations of the effects of diets containing large fractions of bread upon the rate of growth and calcification in rats, and (b) a comparative study of bread and other sources of carbohydrate upon man's energy metabolism.

In the study of the effect of high percentage bread diets on growth and calcification in rats, observations were made on animals confined to the experimental diets during a period in their life when growth and calcification are normally rapid and at a time when any deleterious effects of a dietary regime would be expected to be most pronounced. Since slight deficiencies in a diet may not manifest themselves at once and since the effects of feeding diets below the optimum may not appear until later, this study was continued through successive generations of animals.

Effects of Diets Containing Large Fractions of Bread upon Rate of Growth of Rats

The experimental diets were planned to include large fractions of bread supplemented by foodstuffs which commonly form a part of the diet of children and adults and which supply the necessary food essen-

tials. White bread was utilized in all experiments, because white bread forms 60% of all the bread consumed. The bread utilized was a popular brand of white bread purchased on the market in New York City, prepared with water and not fortified with added vitamins. In preparing this bread for incorporation in the experimental diets, the bread was broken up into small pieces, dried at a temperature below 60° and pulverized. The composition of the three experimental diets is

TABLE I

COMPOSITION OF EXPERIMENTAL BREAD DIETS

Constituents	50% bread diet	60% bread diet	70% bread diet
	Per Cent by Weight		
Dried ground bread	50	60	70
Whole milk powder	40	30	20
Dried yeast (non-irradiated)	5	5	5
Butter fat	2	2	2
Spintrate	1	1	1
Sodium chloride	1	1	1
Dried egg	1	1	1

given in Table I. Originally, in all diets, only brewers' yeast was used, but in the latter part of the experiments dried bakers' yeast was substituted for the brewers' yeast.

It will be observed that there are only two variables in the experimental diets, *i.e.*, the amounts of bread and whole milk powder. Equivalent amounts of the other constituents were included in each of the three diets.

Young albino rats from the stock breeding colony (Wistar strain) were placed at weaning age on the experimental diets. Two breeding lots, each lot including one male and two females, were put on each of the three diets. Litter mates were distributed equally among the breeding lots.

The original breeding lots assigned to the experimental diets have been called first generation animals. Average growth curves are given in Figure 1 for only the males of this group of rats. Since all of the females of this generation were used as breeders, average growth curves for the females have been omitted. Rats born of the first generation animals were divided into two groups, one group included males and females mated in breeding lots; in the other group, rats were sacrificed at different stages of growth for the study of calcification. This procedure of allotting animals into two groups was followed also for the third generation of animals.

Figure 1 also presents the average growth curves for rats of successive generations on each of the experimental diets. The average rates of growth have been calculated only for those rats not assigned to breeding lots but utilized for studies of calcification. Since some of the rats were sacrificed at 56 days, some at 91 days, and some at 119 days of age, the curves represent averages for decreasing numbers of animals during these successive periods of growth. The total number of rats for which average growth curves are represented by the curves in the chart, and the number sacrificed at each of the periods of growth

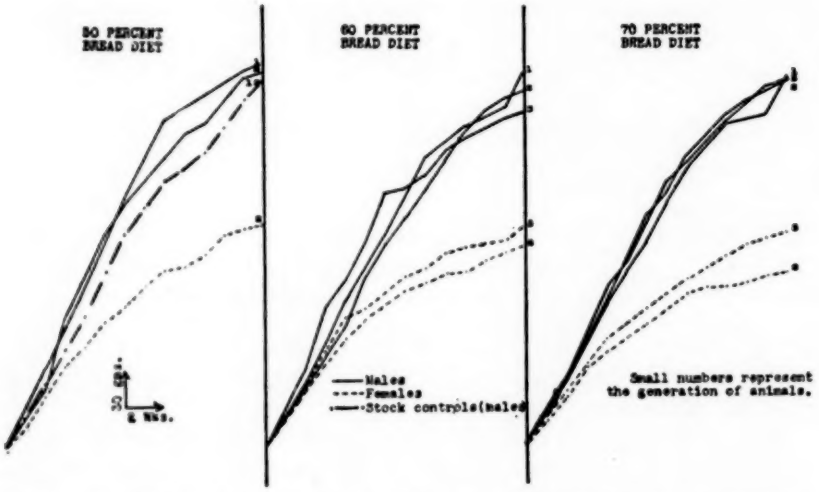

Fig. 1. Growth curves of rats on each of the three bread diets during successive generations.

are recorded in Table II. The curves in Figure 1 indicate that varying the amount of bread in the diets from 50 to 70% had no evident effect upon the rate of growth of either males or females maintained on the experimental diets from 28 to 119 days of age. Data for rates of growth summarized in Figure 1 indicate that these experimental diets containing large fractions of bread demonstrate no deficiencies in growth-promoting factors, nor, on the other hand, did they produce excessive gains in weight for two generations on the 50% bread diet and for three generations on the 60% and 70% bread diets. All growth curves for male rats on the three diets parallel both in rate of growth and in total gain in weight the average curve for male rats on the stock breeding diet (13 M/2).

TABLE II

CALCIUM OF FEMURS OF RATS ON EACH OF THE THREE BREAD DIETS DURING SUCCESSIVE GENERATIONS COMPARED TO CONTROLS ON THE STOCK DIET

Groups	Generation	Age in days	No. of rats	Mean of weight of rats (Grams)	Per cent Ca. in green femur		Per cent Ca. in dried femur	
					Mean	P.E.	Mean	P.E.
Stock		56	5	175	9.3	± 0.58	14.9	± 0.67
Controls (diet 13 M/2)		91	4	226	12.6	± 0.44	17.6	± 0.90
		119	6	251	14.2	± 0.30	19.8	± 0.31
50 Per	2	56	5	151	7.9	± 0.49	14.4	± 0.82
cent bread	2	91	5	195	14.8	± 0.52	21.7	± 0.64
diet	2	119	5	247	15.2	± 0.30	21.1	± 0.49
60 Per	2	56	6	164	9.2	± 0.25	16.6	± 0.67
cent bread	2	91	10	228	13.9	± 0.22	21.2	± 0.16
diet	2	119	8	271	15.8	± 0.18	20.2	± 0.31
60 Per	3	56	6	161	9.5	± 0.27	17.1	± 0.25
cent bread	3	91	6	259	13.3	± 0.22	19.8	± 0.23
diet	3	119	3	262	14.3	± 0.30	19.5	± 0.28
60 Per	4	56	1	190	8.4		16.3	
cent bread	4	91	2	247	13.8	± 0.20	22.0	± 1.03
diet	4	119	2	255	16.4	± 0.53	20.6	± 0.20
70 Per	2	56	5	152	9.1	± 0.37	16.6	± 0.49
cent bread	2	91	5	239	14.1	± 0.49	19.4	± 0.40
diet	2	119	3	291	15.1	± 0.58	22.7	± 0.58
70 Per	3	56	4	168	8.6	± 0.71	15.8	± 0.47
cent bread	3	91	7	225	11.9	± 0.41	18.4	± 0.29
diet	3	119	6	289	14.4	± 0.39	20.8	± 0.08

Effects of Diets Containing Large Fractions of Bread upon the Deposition of Calcium in the Bones of Rats

It is well recognized that diets of excellent nutritive values producing in experimental animals normal gains in weight and every appearance of good health in successive generations, on the other hand, as sources of calcium and phosphorous may be inadequate to enable the body to retain these minerals as rapidly as the optimum development of bone requires in growing animals of succeeding generations and also for the retention of calcium in the mature animals.

In order to observe the effects of the diets containing large fractions of bread upon the retention and utilization of the calcium of the diet, determinations of calcification in the experimental animals were made at periods in their lives when both growth and calcification are proceeding at a rapid rate and at times when any deleterious effects of the diets might be expected to be most pronounced.

TABLE III

CALCIUM AND PHOSPHORUS CONTENT OF BREAD DIETS

Bread in diet	Calcium	Phosphorus	Ratio Ca/P.
	%	%	
50% bread	0.63	0.35	1.8
60% bread	0.51	0.37	1.4
70% bread	0.49	0.26	1.6

Table III presents the analytical data for calcium [2] and phosphorus [2] of the diets and the ratios of calcium to phosphorous. These ratios for each of the three diets do not vary significantly, and all of them are within the range considered optimal for calcium retention, namely, one or two parts of calcium to one of phosphorous.

Some of the animals were sacrificed at 56 days, some at 91 days and some at 119 days. Their femurs were removed and weighed in the green state and in the dry state, and then ashed. Calcium determinations in the ash were made by the method of McCrudden (1911).

Table II presents the mean figures and also the probable errors of the mean for the calcium reported both in terms of per cent of green femurs and of dry femurs. The results for the rats on the experimental bread diets are compared with similar data for rats on the stock diet.

Because all of the rats of the first generation were utilized for breeding, no figures are reported for the calcium in their femurs. At the present time, data for calcium of the rats' femurs are available for only the second generation of animals on the diet containing 50% of bread. Analyses have been completed and are reported for the second and third generations of rats on 70% bread diet, and for the second, third, and fourth generations of animals fed diets of which 60% was bread.

Figure 2 summarizes the results of the studies of calcification for successive generations of rats maintained on the three experimental diets. The columns represent the mean values for calcium in terms of per cent of green femurs. The black portion of the column gives the calcium content of the femurs at 56 days of age, the shaded portion of the column the increment observed at the end of 91 days, and the white portion of the column the increment observed at the end of 119 days. The small figures at the tops of the columns designate the generations of rats. This chart summarizes all of the data now available for the calcium of rats femurs compared to the control animals on the stock diet. Summary of all of the data, as presented here, indicates no sig-

[2] In making determinations for calcium and phosphorus the Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists were used; for calcium, article 36, page 287; and for phosphorus, article 10, page 16.

nificant difference in the degree of calcification of the femurs of the groups of rats on the experimental diets either when compared among themselves or with the controls. For each of the experimental diets

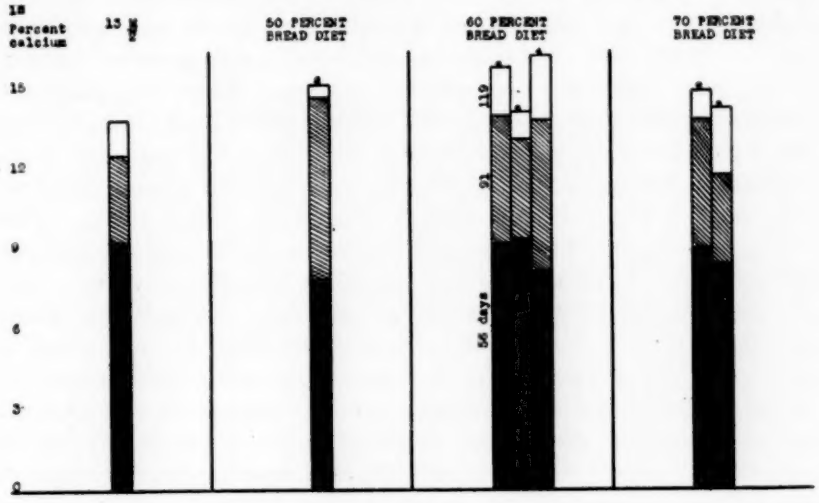

Fig. 2. Percent of calcium in green femurs.

the rats show approximately similar amounts of calcium in the femurs at the corresponding periods of life observed in these experiments. Also when rats are maintained upon diets containing a high fraction of bread, *e.g.*, the 60% bread diet for four generations, the young animals show a normal ability to utilize and deposit calcium and the mature animals to retain their calcium. Hence, under the conditions illustrated in these experiments, a large fraction of bread in the diet does not produce any evidence of inhibition of calcification in the young and also no evidence of decalcification in the mature animals in successive generations.

Tolerance for the Carbohydrates of Bread

Bread, along with other grain products, is classified generally as a food for the support of energy. In an extended series of dietary studies made at the State University of Maine (quoted by Sherman, 1933, p. 325) it was found that breadstuffs and grain products furnished 40% of the fuel value of the average diet. Since the greater part of the fuel value of bread is derived from its carbohydrates, a comprehensive study of the rôle of bread in man's diet should include biologic assays of the availability of the bread carbohydrates and determinations of the tolerance of the average normal human subject for these carbohydrates.

Results of assays of the availability of bread carbohydrates are given by Laug *et al.* (pages 356–364 of this issue of CEREAL CHEMISTRY).

In order to determine the comparative tolerances of human subjects for carbohydrates in bread and for sugars, the procedure described by Killian (1920) was adopted except for the fact that all tests were made after a night's fast. Normal, male adults whose ages varied from 22 to 36 years were the experimental subjects. After emptying their bladders by voluntary voiding, the subjects drank 150 cc. of water. At the end of one hour, the control hour, the total volume of urine in the bladder was collected and a specimen of blood was drawn (by venipuncture) to which an anticoagulant was added (John, 1926). Then the subjects received the bread (190 g. containing 100 g. of carbohydrate and 60 cc. water) or one of the sugars (100 g. in 250 cc. water). Every hour thereafter for three hours, specimens of blood were drawn and the total volume of urine secreted during the hour was collected. Analyses of blood for sugar were made by the method of Folin and Wu (1920). Benedict and Osterberg's (1921) method was adopted for determinations of urine sugar. Throughout the experimental periods, each subject remained at rest, reclining on a couch. Experiments with the different sources of carbohydrate were made on each subject on consecutive days.

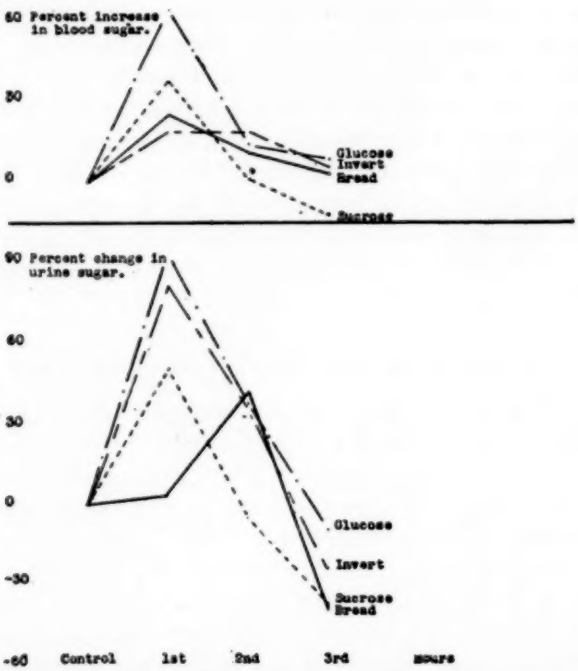

Fig. 3. Average changes in the sugar of the blood and urine after bread and sugars.

Average curves representing variations in blood sugar and sugar excreted in the urine after the consumption of bread or of the sugars are presented in Figure 3. The curves marked bread are averages of results for 6 subjects; those marked sucrose are averages of results for 4 subjects, and those labelled glucose and invert sugar are based upon results for 2 subjects for each of these sugars.

Curves for blood sugar represent average percentage variations in concentrations of sugar in the blood from the levels of the control hours and the curves for urine sugar give the average percentage variations in amounts of total sugar per hour from the quantities excreted during the control hours.

An estimate of a subject's tolerance for carbohydrate should be based upon both changes in blood sugar and in urine sugar for corresponding periods of time after consumption of the carbohydrate. It will be noted that of the four sources of carbohydrate, bread produces the minimum increases in both blood sugar and sugar of the urine. Although invert sugar effects no greater hyperglycemia than does the bread, this sugar enormously increases, *i.e.*, 81% above the control level, the rate of sugar excretion in the urine during the first hour after its consumption. Results of the experiments, summarized in Figure 3, indicate that the experimental subjects exhibit the minimum tolerance for glucose and the maximum tolerance for the carbohydrates supplied in the form of bread.

TABLE IV

COMPARATIVE EFFECTS OF BREAD AND SUGARS, SUPPLYING EQUIVALENT AMOUNTS OF CARBOHYDRATES, ON HEAT PRODUCTION

Subject	B.M.R. Percent deviation from average normal [1]	Food	Fuel value of food calories	Duration of period in hours	Increment of heat—calories	Average hourly increment of heat—percent of basal level
F. D.	— 1	bread	496	3	27	12.5
F. D.	— 4	bread	496	3	26	12.3
T. C.	— 2	bread	496	3	36	17.0
T. C.	0	bread	496	3	45	20.0
L. G.	—10	bread	496	3	25	12.6
J. F.	0	bread	496	3	38	15.3
F. D.	0	sucrose	400	3	11	4.9
J. F.	0	sucrose	400	3	16	6.8
A. M.	+ 5	sucrose	400	3	25	10.0
C. M.	— 6	sucrose	400	3	20	7.8
A. M.	+ 2	glucose	400	3	19	7.9
C. M.	—13	glucose	400	3	11	5.3
A. M.	—12	invert sugar	400	3	14	6.7
C. M.	— 9	invert sugar	400	3	4	1.8

[1] Du Bois normal standards as modified by Boothby and Sandiford.

The comparative effects of equivalent amounts of carbohydrates, supplied either in the form of bread or sugars upon heat production were assayed in a series of 14 experiments on 6 subjects reported in Figure 4 and Table IV. Total heat production in calories per hour were calculated from figures for oxygen consumption and respiratory quotients estimated during the last 10 minutes of the control hour and of each hour after consumption of the foods. All experiments were made in the morning after a fast of 15 hours and throughout the experimental periods, the subjects remained at rest. Under basal conditions, *i.e.*, during the control hours of the experiments reported, the experimental subjects exhibited metabolic rates varying from 13% below, to 5% above the average normal standards. See Hawk and Bergeim (1931).

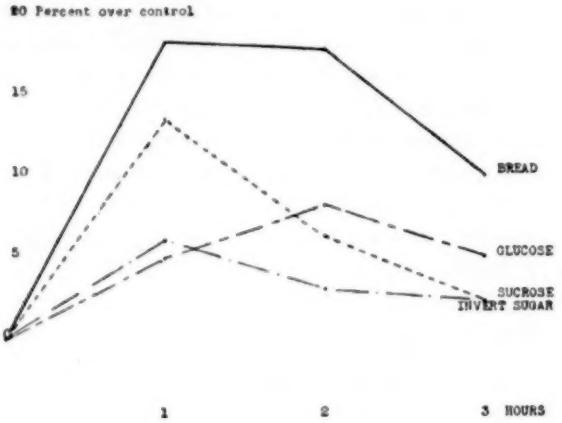

Fig. 4. Comparative effects of bread and sugars on heat production.

Percentage increases in heat production above the levels of the control hours have been averaged for each hour after feeding of bread and the three sugars. These average results are presented in the curves of Figure 4 which represent the comparative specific dynamic actions of these four sources of carbohydrate under the experimental conditions described.

The total increments in heat production for the experimental periods have been calculated as average percentage increases per hour above the basal levels. In 6 experiments with quantities of white bread, supplying 100 g. of carbohydrate, and a total of 496 calories, the average increase in heat production per hour for a three-hour experimental period varies from 12.3 to 20.0% of the basal level; for 8 experiments with sugars, supplying a total of 400 calories, the average increase in heat

production per hour varies from 1.8 to 10.0% of the basal level. Because the quantities of bread and of sugar consumed had different caloric values, the increases in heat production have been calculated as fractions of the fuel value of the food consumed. During experimental periods of three hours, the average figure for extra heat production stimulated by the consumption of bread is 6.6% of the fuel value of the bread; the average figure for the sugars is 3.7% of their caloric values.

From data for nitrogen excreted in the urine respiratory quotients and total heat production in the experiments described above, partitions of total calories among calories from protein, carbohydrate and fat have been calculated. (See Du Bois, 1927.) Because sucrose has produced the maximum average specific dynamic action of the sugars tested, a comparison is reported here of calories for one subject, F. D., after the consumption of bread and after the consumption of sucrose. The comparative experiments were made on consecutive days. On the day of the bread experiment, during the control hour, the subject showed a respiratory quotient of 0.84 and his heat production was 73.2 calories. During the control hour, on the day of the sucrose experiment, this subject's respiratory quotient was 0.78 and he produced 74.1 calories. Hence, it is apparent that on both days, the subject's basal metabolic rates were equivalent (average normal and 1% above average normal).

TABLE V

COMPARATIVE EFFECTS OF BREAD AND SUCROSE ON TOTAL HEAT PRODUCTION AND PARTITION OF CALORIES

Subject F. D.

Time in hours	Material fed	Calories per hour			
		Total	From protein	From fat	From Carbohydrate
Control		73.2	14.3	31.2	27.7
	190 g. bread				
First		82.4	11.0	40.0	31.4
Second		89.0	12.3	27.7	49.0
Third		73.9	13.1	28.0	32.8
Fourth		72.0	11.5	30.3	30.2
Control		74.1	17.4	43.8	12.9
	100 g. sucrose				
First		82.3	12.9	2.2	67.2
Second		78.8	16.9	16.0	45.9
Third		72.2	18.6	21.0	32.6
Fourth		71.3	17.5	22.8	31.0

Data for partition of calories are presented in Table V and in Figure 5. No significant changes in the fractions of calories from protein are observed either after bread or sucrose. The fraction of calories derived from fat oxidation decline rapidly from 59 to 2% of the total during the first hour after the intake of sucrose. However, following the consumption of bread, the fat calories rise during the first hour and then decline to their lowest level, 31% of the total at the time of the maximum heat production; and for the last two hours of the experimental period, the fat calories form 38 and 40% respectively of the total heat produced. It is significant to note that the extra heat production observed after the consumption of bread bears no demonstrable relation to the nitrogen excretion in the urine during the experimental period.

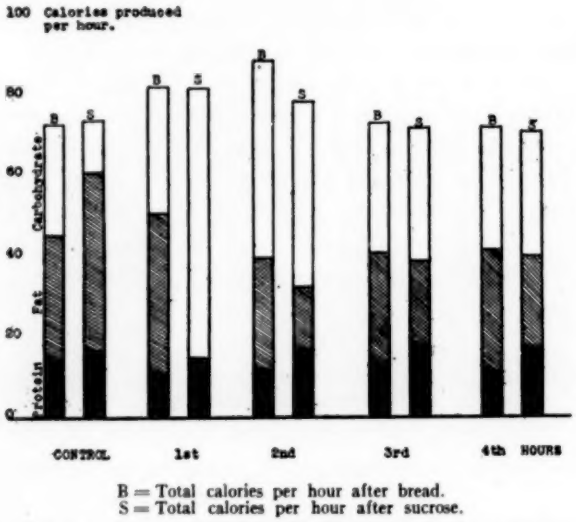

Fig. 5. Partition of calories produced per hour after bread and after sucrose.

A decision concerning the inclusion in the diet or elimination from the diet of a food which is a rich source of carbohydrate should be based not solely upon its chemical composition and calculated caloric value but also upon its characteristic effect on the consumer's metabolic rate.

The scope of this communication does not permit a discussion of the mooted question of the significance of the specific dynamic actions of foods in abnormal energy metabolism. However, for the normal subject at rest, the specific dynamic actions of foods serve as pacemakers of energy metabolism without relation to the estimated fuel values of the foods.

Within the limits of the experiments described, bread has produced

effects upon the subjects' energy metabolism not shown by the three sugars tested. Bread not only has exhibited a greater specific dynamic action but also it has effected a more uniform distribution of heat production between carbohydrate and fat.

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VITAMIN B (B_1) IN BREAD AS AFFECTED BY BAKING [1, 2]

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As soon as the complex nature of vitamin B was established some five or six years ago it became obvious that new determinations of its diverse constituents would have to be made. At least two B vitamins are now recognized, B or B_1 and G or B_2. The former is the antineuritic substance, known to be susceptible of destruction at high temperatures and indeed separable from the more stable B_2 by this very property. The distribution of these two vitamins in natural foods is a question of great practical importance since each is indispensable to good health if not to life and apparently they are not interchangeable with each other in the animal economy. Determinations of the vitamin B complex content of foods such as are now commonly found in vitamin tables are consequently of little value and must be replaced by vitamin B_1 and B_2 figures separately determined.

Our former comfortable feeling that the American diet is well supplied with vitamin B is not adequately available. In particular there is danger of a less than optimum vitamin B_1 content. This is due apparently to our use of highly milled cereal products, autoclaved vegetables and fruits and to the rather sparse occurrence of this vitamin in most fresh fruits and vegetables, milk and meat.

Since bread is the chief form in which grain foods are eaten and since these foods are the chief source of vitamin B_1 any inquiry into the conditions affecting this vitamin in bread is of nutritional as well as of economic interest. The vitamin value of the raw materials as well as of bread baked under varying conditions should be known in order to judge the amount of destruction produced by the baking process.

Very little could be found in the literature either upon the B_1 content of bread or the amount of its destruction in baking. Chick and Hume (1917) made some baking experiments in 1917 following their work on the stability to heat at 100° C. of the antineuritic property of wheat germ and concluded that this quality is not destroyed in bread,

[1] Presented before the Biological Section at the 88th meeting of the American Chemical Society at Cleveland, Ohio, September 10–14, 1934.

[2] The study was supported by a grant from Standard Brands Inc.

since the internal temperature of the loaf rises to only 100–101° C. and the baking time need not exceed one hour. Hashitani and Sako (1932), using pigeons fed an exclusive diet of white bread, came to the conclusion that this food is deficient in antineuritic activity but that the addition of 0.3 or 0.4% dry brewery yeast to the formula produced bread of adequate vitamin B value for the protection of the birds. They state that such an addition does not spoil the flavor and appearance of the bread. They also noted the internal temperature of the loaves during baking to be no higher than 90 to 100° C. and this maximum to be maintained only 10 to 20 minutes. They drew the conclusion that little if any loss of the antineuritic property of bread is produced by the baking process.

Other suggestions for the improvement of the vitamin B value of bread have been made by Krogh (1931) and by Eyre (1933), in each case through the medium of the addition of extra baker's yeast. Dr. Krogh calculated that an increase of the yeast to five times the usual amount would result in the production of a bread which would provide in 580 g. (about 20 oz.) sufficient vitamin B for the needs of the average adult. This suggestion was prompted by a survey of Danish foods which indicated a probable inadequacy of vitamin B in the diet of a large part of the population. Miller (1931) with a similar condition in mind has suggested the incorporation of rice bran in bread used in Hawaii and reports that about 20% of the vitamin B content of the bran is destroyed during baking. Wheat germ has been used to enrich the well known Hovey's bread in England for some 25 to 30 years and its inclusion as well as that of rice polish in yeast bread and rolls has been advocated for some years by the Bureau of Home Economics of the United States Department of Agriculture. Successful feeding experiments with underweight children fed wheat germ rolls in this laboratory (Morgan and Barry, 1930) stimulated our further inquiry into the vitamin B_1 value of all varieties of bread.

The questions asked are these: 1. What is the average content as well as the maximum loss of vitamin B_1 in whole wheat, white and rye breads during the baking process? 2. What effect upon the loss, if any, may be produced by oven temperature variation? 3. Does size of loaf influence the loss? 4. Is the crust more likely to lose vitamin B_1 than the crumb? 5. Is it possible materially to increase the vitamin B_1 of the white loaf without changing excessively its appearance and flavor?

Feeding Methods

In the attempt to obtain an answer to these questions bread was baked under the varying conditions stated and was fed as the sole

source of vitamin B_1 to young rats and to mature pigeons. Substantially the method of Chase and Sherman (1931) was used in the rat experiments and that of Salmon (1927) in the pigeon series. The basal diet fed the rats consisted of extracted casein 18, Osborne and Mendel salt mixture 4, butterfat 8, codliver oil 2, autoclaved yeast 15, cornstarch 53. When properly supplemented with vitamin B_1 this diet allows normal growth and when no such supplement is given the animals lose weight and die within three to four weeks. The rats when placed on the diet without vitamin B_1 supplement at 28 days of age within two weeks showed stationary weight. This was taken to indicate depletion of their vitamin B_1 reserves and the test material was then regularly fed for eight weeks. The gain in weight during these eight weeks was taken to represent the relative vitamin B_1 content of the material under test. Usually three levels of test dosage were used. The International Standard was used for a reference group at intervals as was also our standard rice polish concentrate.

The pigeons were fed by hand daily 8 g. of autoclaved rice and 8 g. of a mixture consisting of casein 20, polished rice 73, agar 2, salt mixture 2, autoclaved yeast 6, and cod liver oil 3. On this diet the birds lost weight and became polyneuritic within a few days, but when an adequate amount of vitamin B_1 containing food was included they maintained or gained weight and remained healthy for an indefinite period. The daily dose of dry bread or grain was given in place of part of the rice. When a bird had been maintained in health for two to four weeks on a given test dose the amount of the dose was decreased by 10% and another feeding period carried out. If the new level was below the maintenance requirement in two to six days the pigeon began to disgorge its food, to lose weight and soon to show the classic symptoms of polyneuritis. In this manner the maintenance level may be rather accurately obtained. A cured bird may be used over and over for such tests. All of the pigeons were standardized by determining their maintenance requirement of a reference supply of wheat germ and for the bread experiments for whole wheat and whole rye as well. Two birds were used to determine the value of the wheat germ and of our standard rice polish concentrate in terms of the International Standard, the acid adsorbate supplied by the League of Nations' Committee on Vitamin Standards. Restandardization of the birds every three or four months has been found to be desirable.

The Bread Making

The bread was baked for the most part according to the formulas suggested by the American Association of Cereal Chemists (1924, 1925, 1927) for use in baking tests with flour. For the pound whole wheat loaf the formula was:

	Grams
Flour	338.0
Yeast	6.7
Salt	6.7
Sugar	13.5
Fat	10.1

While for the white bread the formula was:

	Grams
Flour	350.0
Yeast	10.5
Sugar	10.5
Salt	5.2
Fat	6.5

For the part whole wheat, whole rye and part rye breads the same formulas were used except that one-half or one-third whole wheat flour and one-third or all rye flour was substituted for corresponding weights of white flour in the white bread formula.

The raisin bread was made according to the following formula:[3]

	Grams
Flour	230
Yeast	7
Salt	3
Sugar	20
Fat	12
Seedless Rasins	150

This formula leaves approximately one-third raisins in the baked loaf or 65% of the flour in the formula.

The same formula was used for a corresponding white bread with raisins omitted.

The milk bread was made by the white flour formula with addition of 4 g. dry skim-milk per 100 g. of flour.

The white, rye, part rye, part whole wheat and other mixed breads were baked in one pound loaves for 25 or 35 minutes in an oven at 446° F. The raisin bread and some of the whole wheat bread was baked for 45 minutes at 350° F.

Both the whole wheat and the whole rye flours were made in the laboratory from the whole grains and the white flour used was a standard patent, family flour. A baker's strong flour was later used but no significant change in antineuritic value of the bread resulted. The mixing and proofing of the doughs were carried out as suggested by the committee on formula and method of the American Association of Cereal Chemists (see Fitz, 1924 and 1925, and Blish, 1927). The loaves in all cases were air-dried and the daily doses weighed out from the dry material. Water determinations accurately made on all the whole wheat, rye and white products, kernels, raw dough and baked

[3] We acknowledge the assistance of Ruth M. Popper in the making and testing of the raisin and milk breads.

TABLE I

VITAMIN B (B_1) VALUE OF WHOLE WHEAT BREAD

Product	Daily dose	Number of rats	Initial (depleted) weight	Gain in 8 weeks	International units per 100 grams	Average
	Grams		*Grams*	*Grams*		
1 lb. loaf baked at 350° F	0.4	6	54	15	110*	
	0.6	19	68	35	171	178
	0.8	9	73	50	184	
1½ lb. loaf baked at 350° F	0.4	6	59	24	176*	
	0.6	5	66	37	181	183
	0.8	8	73	50	184	
1 lb. loaf baked at 446° F	0.4	6	63	6		
	0.6	6	69	34	167	166
	0.8	5	79	45	165	
1½ lb. loaf baked at 446° F	0.4	6	60	15	110*	
	0.6	6	69	44	215	216
	0.8	5	67	59	217	
1 lb. loaf commercial whole wheat bread	0.6	6	78	9		
	0.8	6	82	46	167	167
Crumb only, all loaves baked at 350° F	0.4	6	58	10		
	0.6	12	59	39	191	199
	0.8	18	87	56	206	
Crumb only, all loaves baked at 446° F	0.4	10	64	14	103*	
	0.6	19	61	41	201	205
	0.8	15	61	57	209	
Crust only, all loaves baked at 350° F	0.4	6	66	7		
	0.6	9	77	20	98	137
	0.8	6	57	48	176	
Crust only, all loaves baked at 446° F	0.4	11	67	1		
	0.6	17	67	19	93	131
	0.8	11	63	46	169	
Raw wheat No. 1	0.4	15	68	18	132*	
	0.6	13	66	38	186	189
	0.8	15	77	52	191	
No. 2	0.4	5	71	−6		
	0.6	12	74	29	142	142
Unbaked dough	0.4	6	76	4		
	0.6	6	68	35	168	190
	0.8	6	69	58	213	
International Standard	0.005	7	79	−4		
	0.010	26	68	34	100	

* Omitted from averages.

breads yielded practically identical figures, 7.9 to 8.8, except in the case of the raisin bread which carried 14% water. The air-dried products with this exception were therefore assumed to be comparable. The fresh breads were also examined for water content and were found to vary very little, 32 to 35% water being present in all cases.

Results

The results of these experiments are summarized in eight tables. Table I shows the relative vitamin B_1 value of the 100% whole wheat bread made from wheat No. 1, to be practically that of the whole grain and of the unbaked dough. No significant loss of vitamin appeared to occur during the baking although the smaller loaves, 1 pound, showed somewhat less growth-promoting value than did the corresponding 1½ pound loaves. A demonstrably lower value of the crust as compared with the crumb of all these loaves was also seen. All the results are expressed in International units as obtained under our laboratory conditions with the vitamin B adsorbate obtained from Dr. E. M. Nelson, custodian in this country of the International vitamin standard preparations.

A word should be said as to the relation of this unit to the well-known Chase and Sherman unit of vitamin B. The latter is defined as the amount of vitamin B which will allow 25 g. gain in body weight in eight weeks by standard rats fed a vitamin B-free but otherwise adequate basal diet. Since our rats grew at an average rate of 34 g. in eight weeks under these conditions when given 1 unit, that is 10 mg. daily of the International standard, the International unit in our hands represents slightly more than one and one-third Sherman units. The variability of the different groups fed this standard is illustrated in Table II, as is also the disproportionally low response obtained when

TABLE II

GROWTH RESPONSE OF RATS TO INTERNATIONAL VITAMIN B (B_1) STANDARD

Lot	Number of rats	Daily dose	Weight at		Gain in 56 days on dose
			28 days	Depletion	
		Mg.	*Grams*	*Grams*	*Grams*
1	4	5	56	75	−8
2	3	5	57	84	1
5	6	10	42	73	37
4	8	10	44	72	29
5	4	10	47	63	55
6	4	10	54	66	24
7	4	10	44	65	26
Average	26	10 (1 unit)	47	68	34 ± 2

a sub-maintenance daily allowance is fed. Thus on 5 mg. of the standard loss of weight usually occurred but on 10 mg. a steady growth at about one-third or one-fourth the normal rate resulted. The same relation may be seen in the bread experiments, 0.4 g. of whole wheat bread usually allowing little or no growth but 0.8 g. about half normal growth. Arithmetical correspondence between size of dose and resulting growth apparently can be expected only in the narrow range of doses producing increases from one-third to fully normal growth rates.

The value in International units was calculated by means of the formula,

$$\frac{W}{34 \times D} \times 100 = \text{number of units per 100 g.},$$

in which W is the average number of grams gain in eight weeks of all the rats on a given daily dose D (in grams) of the substance tested and 34 represents average gain on 1 unit daily of the International standard. Only results of trials in which reasonable, measurable growth took place, 20 g. or more in the eight weeks, can be included in these calculations. All trials are calculated in Table I to illustrate the method by which the averages were obtained.

TABLE III

THE VITAMIN B (B_1) CONTENT OF RYE BREAD

Product	Daily amount fed	Number of rats	Initial (depleted) weight	Gain in 8 weeks
	Grams		*Grams*	*Grams*
Whole rye bread	0.4	6	74	3
	0.6	7	73	13
	0.8	10	68	33
Whole rye dough, unbaked	0.4	6	75	−3
	0.6	5	76	14
	0.8	8	79	30
One-third rye bread	1.0	15	71	20
	1.5	19	80	37
One-third rye dough	1.0	5	73	4
Whole rye grain	0.4	7	72	−4
	0.6	7	74	12
	0.8	7	73	37

In Table III in which are shown the rye and rye bread values it may be seen that both the grain and the bread are lower than wheat in vitamin B_1. The first wheat sample which we used was nearly twice as rich as the rye but the second wheat sample was only slightly better than the rye. It is recognized of course that various samples of these grains vary considerably in this respect. Apparently no loss of vitamin was incurred in the baking of the rye bread.

TABLE IV

VITAMIN B (B_1) CONTENT OF WHITE BREAD

Product	Daily amount fed	Number of rats	Initial (depleted) weight	Gain in 8 weeks
	Grams		*Grams*	*Grams*
White bread	2.0	7	69	7
	2.5	10	66	26
	3.0	12	70	43
	4.0	9	58	80
	5.0	6	59	77
	6.0	5	65	67
Composite sample commercial white bread	2.5	10	74	28
Unbaked white dough	3.0	12	77	39

In Table IV the results with white bread show that this bread is far from lacking in vitamin B_1 but has instead about one-fourth the value of the whole wheat product.

A composite sample of commercial white bread, made from one pound loaves of six well-known brands yielded almost the same results as did the laboratory-made white bread. The white bread made as controls for the raisin and skim-milk breads may be seen in Table V to

TABLE V

VITAMIN B (B_1) CONTENT OF MIXED BREADS

Product	Daily amount fed	Number of rats	Initial (depleted) weight	Gain in 8 weeks
	Grams		*Grams*	*Grams*
One-third whole wheat	1.0	7	72	5
	1.5	15	80	21
One-half whole wheat	0.8	3	74	5
	1.0	15	77	2
	1.5	13	79	35
One-half whole wheat, crust only	1.5	9	79	15
crumb only	1.5	9	80	32
Commercial "wheat" bread	1.5	8	83	44
White bread, containing 20% wheat bread	0.5	8	74	25
	0.8	10	85	28
White bread, containing 4% dry skim-milk	1.5	12	78	0, 4 died
	2.0	12	71	10, 4 died
	2.5	4	63	25
	3.0	4	65	44
Same, without skim-milk	1.5	10	86	−14, 5 died
	2.0	9	96	−21, 5 died
Commercial "milk" bread	1.5	8	85	−9, 1 died
	2.0	8	85	16
White bread, containing 33% raisins	1.5	9	65	14
	2.0	20	72	37
	2.5	14	74	43
Same without raisins	1.5	9	68	5, 2 died
	2.0	9	65	28
	3.0	29	69	50

have almost the same growth promoting value as the other white breads.

The addition of 20% wheat germ was considerably effective, since it trebled the vitamin B_1 value of the bread without greatly changing the appearance.

TABLE VI

VITAMIN B (B_1) CONTENT OF ADDED INGREDIENTS

Product	Daily dose	Number of rats	Initial (depleted) weight	Gain in 8 weeks	International units per 100
	Grams		*Grams*	*Grams*	*Grams*
Fresh compressed yeast	1.00	6	64	24	
	1.50	6	75	48	82
Wheat germ (3 samples)	0.10	14	63	25	
	0.15	15	66	41	770
Rice polish concentrate (tiki-tiki)	0.03	14	57	41	
	0.06	15	58	102	
	0.10	8	75	140	4380
Seedless raisins	1.50	5	57	28	
	2.00	6	73	31	50
	2.50	9	74	42	
International standard	0.01 (1 unit)	26	68	34	10000

The various added ingredients used in the mixed breads (Table V) were assayed separately and the results are shown in Table VI. It will be noted that the raisins have a little more vitamin B_1 content than the dry white bread and consequently it is not surprising that the raisin bread had a little more vitamin value than the white bread.

The milk breads, both composite commercial samples containing the skim-milk specimens, were no different from the ordinary white bread in this respect.

The results obtained with the pigeons confirmed the data found by rat growth. A summary of figures obtained on the whole wheat and whole rye breads and grains is given in Table VII. No loss can be said to be occasioned by baking except in the crust of breads baked at 446° F.

A summary of the vitamin B_1 content of all the breads studied is given in Table VIII in terms of International units as defined in this paper, and on air-dry and fresh basis. The part wheat breads made from wheat sample No. 2 and the commercial "100% wheat" breads show a lower value than the original laboratory-made whole wheat breads made from wheat sample No. 1. It is fair to assume then that such breads will vary usually at least within these limits, 98 to 130 Inter-

national units per 100 g. or 40 to 53 Sherman units per ounce fresh weight.

TABLE VII

PIGEON MAINTENANCE (VITAMIN B_1) VALUE OF BREADS

Product	Number of birds	Daily minimum required for protection			Loss of vitamin B_1 in baking
		Whole wheat or rye	Equivalent in bread	Bread required	
		Grams	*Grams*	*Grams*	%
Whole Wheat Bread					
1 lb. loaf baked at 446° F.	4	4.5	4.9	4.9	0
1½ lb. loaf baked at 446° F.	3	4.3	4.7	5.0	6
1 lb. loaf baked at 350° F.	3	4.4	4.9	5.0	2
1½ lb. loaf baked at 350° F.	3	4.2	4.7	4.6	0
Crust only baked at 350° F.	6	4.0	4.3	4.5	5
Crust only baked at 446° F.	6	4.2	4.7	5.3	13
Crumb only baked at 350° F.	7	3.9	4.3	4.3	0
Crumb only baked at 446° F.	6	4.2	4.4	4.7	7
Whole Rye Bread	5	3.8	4.2	4.2	0

The rye bread made from only one rye sample had a lower B_1 content than had any of the whole wheat breads, 84 International units per 100 g. or 34 Sherman units per ounce. The part rye and part wheat

TABLE VIII

SUMMARY OF VITAMIN B (B_1) CONTENT OF BREADS AND GRAINS

Product	International units per 100 g.	
	As fed air-dry	Fresh weight
Whole wheat bread (from wheat No. 1)	186	130
Same, crust only	134	94
Same, crumb only	196	137
Commercial whole wheat bread	167	117
Whole wheat grain No. 1	189	189
" " " " 2	142	142
One-half whole wheat bread (from wheat No. 2)	68	48
One-third whole wheat bread (from wheat No. 2)	48	34
Commercial "wheat" bread	86	60
White bread	42	30
White bread, 20% wheat germ	125	88
White bread containing 4% dry skim-milk	42	30
Commercial "milk" bread	42	30
White bread, containing 33%	54	38
Whole rye bread	121	84
One-third rye bread	65	45
Whole rye grain	136	136

breads varied as might be expected with the amount of whole grain included, showing improvement over the white breads of from 13 to 100% or a vitamin B_1 content of 14 to 24 Sherman units per ounce as compared with the usual 12 units per ounce of white bread.

Conclusions

In conclusion we may now attempt to answer so far as these data will allow the five questions propounded.

Practically no loss of vitamin B_1 occurs in whole wheat, rye and white breads during the baking process. These breads contain 98 to 130, 84 and 30 International units per 100 g. respectively, or in Sherman units, 40 to 53, 34 and 12 per ounce of fresh bread.

Oven temperature variation as represented by 350° F. and 446° F. has no consistent effect upon the vitamin B_1 content of bread.

Size of loaf has a small but demonstrable effect in that 1½ pound loaves baked under identical conditions with 1 pound loaves have slightly the higher vitamin B_1 content.

The crust of the whole wheat bread was found to be definitely lower in vitamin B_1 than the crumb, the maximum lowering being about 35% as shown by rat growth method. Only 13% loss was noted however by the pigeon maintenance method. This result is not unexpected since the temperature attained by the crust must be considerably higher than that of the rest of the loaf.

The addition of 20% wheat germ nearly trebled the vitamin value of the bread but produced a loaf of somewhat small volume, poor texture and yellowish color. Such a loaf is however lighter and smoother than most whole wheat breads.

Addition of raisins, 65% of the flour weight, increased the vitamin value of the bread about 25% but the usual 4% skim-milk powder addition did not change the vitamin B_1 content of white bread. Bread sold as " milk bread " was likewise found to be the same as ordinary white bread in this regard.

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A COMPARATIVE STUDY OF THE BLISH AND SANDSTEDT AND A MODIFIED RUMSEY PROCEDURE FOR THE ESTIMATION OF DIASTATIC ACTIVITY[1]

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Introduction

A knowledge of the diastatic activity of flour is a valuable aid in indicating the soundness of the wheat from which it was milled, in the interpretation of experimental baking results, and in predicting its behaviour under any particular set of commercial baking conditions. For some years this determination has been a matter of routine laboratory practice in many of the flour studies with which the senior author has been associated, the technique suggested by Rumsey (1922), with certain modifications, being employed. The modifications introduced consisted of conducting the diastasis at 30° C. to conform with the fermentation temperature used in test baking, and employing the citrate-HCl buffer solution suggested by Malloch (1929) to control acidity. The reducing sugars present after one hour's autolysis were determined by the copper reduction method of Quisumbing and Thomas (1921), direct weighing of the cuprous oxide being resorted to. Initially, "blank" determinations were made to correct for the reducing sugars originally present in the flour but when it was found that the values were of a low order and approximately constant, they were dispensed with and the anhydrous maltose, equivalent to the total cuprous oxide found after one hour's diastasis, was expressed as the diastatic activity. Blish, Sandstedt, and Astleford (1932) have presented evidence that the blank determination is ordinarily unnecessary and previously Kent-Jones (1927) indicated a similar belief.

Despite the elimination of the blank determination, the necessity of centrifuging the flour suspensions with the frequent occurrence of cloudy extracts, coupled with the time and labour involved in the determination of the reducing sugars by the method employed, is a serious disadvantage for routine determinations. The modification of

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Hagedorn and Jensen's (1923) method, recently proposed by Blish and Sandstedt (1933) for the determination of reducing sugars, is a great improvement in these respects and, since its publication, the procedure they recommended has been employed in this laboratory. Determinations have now been made on several hundred flours and the method was found to be very convenient and to give good agreement between duplicates. In view of the importance of diastatic activity determinations, however, it was deemed desirable to undertake a critical comparison of the results obtained by the procedure formerly employed with those given by the improved method of Blish and Sandstedt (1933).

Experimental

The experimental material consisted of a series of 89 samples, comprising both experimentally and commercially milled flours, the diastatic activity of which ranged in fairly progressive increments from 70 to 317 units, as determined by the improved method. Four subsamples of each flour were employed, two of which were treated according to the method outlined by Blish and Sandstedt (1933) and the remaining two by the modified Rumsey procedure formerly employed in this laboratory.

In both cases, the weights of the flours taken for analysis were adjusted to a 13.5% moisture basis, the volume of buffer solution added in the instance of the improved method being varied to give a total of 46 cc. solution on the basis of flour containing 13.5% moisture.

In the regular laboratory method, 10 g. of flour on a 13.5% moisture basis, and 100 cc. of the sodium acetate-acetic acid buffer solution (pH 4.61) suggested by Blish and Sandstedt (1933) were employed, the diastasis being conducted at 30° C. With the exception of these modifications, the method of diastasis and determination of reducing sugars corresponded to that detailed in the A.A.C.C. Book of Methods (1928); as recommended, the reducing sugars were determined by the Quisumbing and Thomas procedure (1921). The cuprous oxide was first obtained by direct weighing, after which it was dissolved in dilute nitric acid and determined by the "volumetric thiosulphate method."

While the conditions of diastasis in the two procedures are similar in regard to acidity, time, and temperature, the concentrations of the flour suspensions differ. In the Blish and Sandstedt procedure, 46 cc. buffer solution and 5 g. flour are employed for diastasis, as compared with 100 cc. buffer and 10 g. flour in the modified Rumsey method. It should also be noted that the volume occupied by the flour is not taken into consideration in the latter method. The flour suspension, after addition of the clarifying agents, is made up to 200 cc. and, after

centrifuging, the reducing sugars determined on an aliquot of the supernatant liquid; while in the improved method, the volume of the flour is not a factor, the total volume of buffer solution and clarifying agents added being 50 cc. and a 5 cc. aliquot of the filtered extract employed for determining the reducing sugars.

The determinations were carried out according to a time schedule and the results expressed in Rumsey units without regard to the reducing sugars originally present in the flours.

In regard to experimental technique, the method suggested by Blish and Sandstedt possesses several advantages. Elimination of the necessity of making the suspensions up to volume and of centrifuging represents a great saving of time and trouble from frothing and cloudy extracts. The determination of the reducing sugars by the ferricyanide method is convenient and rapid. By carrying out the reduction of the ferricyanide in boiling water, the second thermostatically controlled water bath called for in the Quisumbing and Thomas bath is not required. In the gravimetric procedure, difficulties in filtering the cuprous oxide through a Gooch crucible frequently occur. The volumetric procedure for determining reduced copper is quite a lengthy process and the end point of the titration is somewhat difficult to judge. In contrast to this, the volumetric determination of the unreduced ferricyanide is very straightforward and the end point is sharp. By the improved procedure, one operator can readily handle twice the number of determinations per day as by the gravimetric copper method. In our experience the volumetric thiosulphate method is more time-consuming than the direct weighing of the cuprous oxide.

Statistical Studies

The data obtained by the three methods under study were first submitted to a variance analysis in order to determine their relative values in differentiating between flours. The total variance was divided into two components, namely, that due to the differences between the pairs of duplicate values, and that due to the differences within pairs. The magnitude of the variance for between pairs depends upon the variability of the means of the duplicate determinations while that for within pairs depends upon the differences within each pair of values, and hence is a measure of the experimental error. The larger the variance between the means of the duplicate determinations, and the smaller the differences within each pair of duplicates, the greater will be the differentiation between the flours. Hence the magnitude of the Z value obtained by comparing these two estimates of variance is a measure of the value of any particular

method in differentiating between flours. From the results of the three variance analyses given in Table I, it will be observed that the

TABLE I

ANALYSES OF VARIANCE FOR DIASTATIC ACTIVITY VALUES

Variance due to	Sum of squares	Degrees of freedom	Variance	Z	5 % pt.	1 % pt.
Blish and Sandstedt Method						
Differences between pairs	636,491.956	88	7232.86	3.1165	0.2487	0.2503
Differences within pairs (Error)	1,264.000	89	14.202			
Total	637,755.956	177				
Modified Rumsey Method (Gravimetric Determination of Reduced Copper by Quisumbing and Thomas Procedure)						
Differences between pairs	891,601.6	88	10,131.8	2.6038	0.2487	0.2503
Differences within pairs (Error)	4,937.5	89	55.478			
Total	896,539.1	177				
Modified Rumsey Method (Volumetric Determination of Reduced Copper by Quisumbing and Thomas Procedure)						
Differences between pairs	872,872.4	88	9919.0	2.5329	0.2487	0.2503
Differences within pairs (Error)	5,564.0	89	62.517			
Total	878,436.4	177				

improved method of Blish and Sandstedt (1933) gives a Z value of 3.1165 as compared with 2.6038 and 2.5329 for the gravimetric and volumetric copper reduction procedures. As these values greatly exceed the 5% and 1% points, they are highly significant. The significance of the differences between all three possible pairs of the above Z values was determined by comparing the differences with their standard errors. Designating the Z values for the "ferricyanide," gravimetric and volumetric procedures as Z_1, Z_2, and Z_3 respectively, the differences between the three pairs of Z values are: $Z_1 - Z_2 = 0.5127$, $Z_1 - Z_3 = 0.5836$ and $Z_2 - Z_3 = 0.0709$, the standard error in each case being $\pm$ 0.1503. Thus the "ferricyanide" procedure gives a much better differentiation between the diastatic

activity of the flours than either the gravimetric or volumetric copper reduction methods, which are of equal value. It should be noted that the spread between the mean diastatic activity values, as shown by the variances for "differences between pairs," is lower for the ferricyanide procedure but this is more than counteracted by the much lower experimental error for this method. The error variances have been compared by means of the Z test, and the variance for the ferricyanide technique was found to be significantly less than that of either the gravimetric or volumetric copper methods, the difference between these being insignificant.

The square roots of these error variances represent the respective standard deviations of the differences between duplicate determinations and these are recorded in Table II, along with the other statistical

TABLE II

STATISTICAL CONSTANTS FOR DIASTATIC ACTIVITY VALUES

	Constants for means of duplicates				Constants for differences between duplicates	
	Means	Standard deviation	Range		Mean	Standard deviation
			Min.	Max.		
Ferricyanide method (x)	172.72	60.14	70	317	3.86	3.77
Gravimetric copper method (y)	177.35	71.23	68	371	7.67	7.45
Volumetric copper method (z)	176.68	70.43	67	369	8.31	7.91

Statistics for Means of Duplicates

Simple correlation coefficients	r	5% point
Ferricyanide method × gravimetric copper method, r_{xy}	0.984	0.206
Ferricyanide method × volumetric copper method, r_{xz}	0.990	0.206
Gravimetric copper method × volumetric copper method, r_{yz}	0.998	0.206

Regression coefficient

Gravimetric copper method (y) on Ferricyanide method (x) $B_{yx} = 1.166$; $t = 51.6$

Linear regression equation

Estimation of diastatic activity (Gravimetric copper method) (y) from Blish and Sandstedt method (x)

$y = 1.166x - 23.955x$; standard error of prediction = $\pm$ 12.7

constants which have been computed. The mean differences between duplicates for the ferricyanide, gravimetric, and volumetric copper reduction values are 3.86, 7.67, and 8.31 Rumsey units, the corre-

sponding standard deviations being 3.77, 7.45, and 7.91 units respectively.

From these standard deviations of the differences between duplicate determinations, the minimum significant differences in diastatic activity for any two flours in the series may be computed for the three methods. Since the standard error of the mean of two determinations equals the standard deviation divided by $\sqrt{2}$, the standard error of the difference between the means of two sets of duplicate determinations equals the standard deviation $(\text{S.D.}/\sqrt{2}) \times (\sqrt{2})$. Taking the level of significance as twice the standard error, a difference between the mean values of any two flours equal to twice the standard deviation of the differences between duplicates would be obtained by random sampling in only 5% of the cases. These values for the ferricyanide, gravimetric, and volumetric copper procedures are 7.5, 14.8, and 15.8 Rumsey units respectively.

Considering now the statistics computed for the means of the duplicates, it will be observed from Table II that the Blish and Sandstedt method gave a mean diastatic activity for the entire series of 172.7 Rumsey units, the corresponding values for the modified Rumsey method being 177.4 and 176.7 for the gravimetric and volumetric copper determinations respectively. Moreover, the standard deviation of the individual means is lowest for the improved method, which is a reflection of the smaller spread in diastatic activity given by this method, the range being from 70 to 317 units or a spread of 247 units, as compared with spreads of 303 and 302 units for the gravimetric and volumetric copper values respectively. While the correlations between the values given by the three methods are all of a high order, that of 0.998 between the gravimetric and volumetric copper values is significantly higher than the correlations 0.984 and 0.990 in which the values by the Blish and Sandstedt procedure are correlated with those given by the gravimetric and volumetric copper procedures respectively. In this connection it should be recalled that the gravimetric and volumetric determinations of reduced copper were made on the same precipitates and hence excellent agreement should be obtained if the two methods give concordant results.

The significance of the difference in mean values yielded by any two procedures may be determined by comparison with its standard error. The standard error of the mean difference between two sets of correlated values is given by the formula

$$\sigma M_{A-B} = \frac{\sqrt{\sigma A^2 + \sigma B^2 - 2r_{AB}\sigma A \sigma B}}{\sqrt{n}},$$

where n = the number of pairs of values involved. The mean differences between the Blish and Sandstedt procedure and gravimetric and volumetric copper methods are 4.63 ± 1.74 and 3.97 ± 1.46 respectively, while that between the gravimetric and volumetric copper values is 0.67 ± 0.43. As the mean difference between the two copper methods does not exceed twice its standard error, they may be regarded as yielding the same absolute values. It has previously been shown that the experimental errors are of the same order and there is therefore nothing to choose between them from the standpoint of reproducibility of results and absolute values obtained.

The improved method, however, gives a significantly lower mean value and a narrower spread between samples than the modified Rumsey method of diastasis and determination of the reduced copper by either the gravimetric or volumetric procedures.

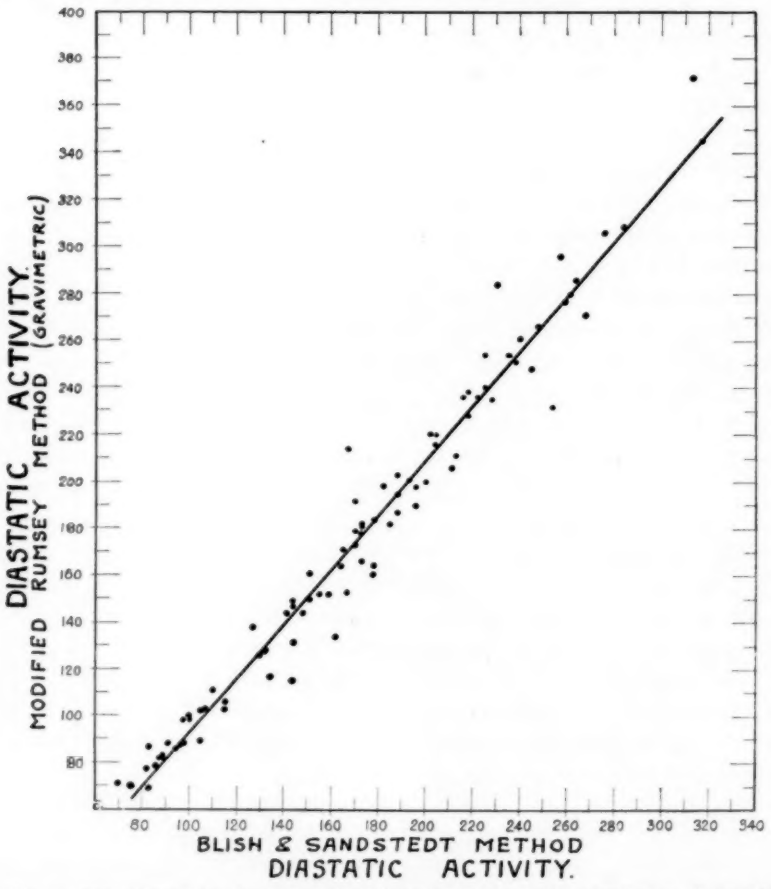

Fig. 1. Dot diagram for the comparative diastatic activity values obtained on 89 individual flours by the methods indicated. The regression line is for the regression of diastatic activity by the "gravimetric copper method" on the values given by the improved Blish and Sandstedt procedure.

The narrower spread is indicated by the regression coefficient $B_{yz} = 1.166$ given in Table II, since if the two procedures gave the same range in values, the regression coefficient would be 1.0. This is also shown graphically in Figure 1, in which the means of the duplicate determinations on the individual flours by the Blish and Sandstedt and gravimetric copper procedures are plotted against each other. It will be noted that with flours of low diastatic activity, the former method tended to give slightly higher values than the latter, while with flours of high diastatic activity, the Blish and Sandstedt improved method gave appreciably lower values in our hands. However, the correlation between the two sets of values is 0.984 and from the linear regression equation given in Table II, the diastatic activity by the Rumsey method may be estimated from the Blish and Sandstedt values with a standard error, for a single predicted value, of $\pm$ 12.7 units. This may be regarded as quite satisfactory and the improved procedure is to be preferred from the standpoints of greater convenience, lower experimental error, and better differentiation between flours.

Summary

A comparative study of a modified Rumsey method and the improved method of Blish and Sandstedt involving a modification of the ferricyanide reduction procedure of Hagedorn and Jensen for the determination of diastatic activity of wheat flour has been made on 89 flours varying in diastatic activity from approximately 70 to 300 units. The modifications in the Rumsey method consisted of conducting the diastasis at 30° C. and the use of a sodium acetate-acetic acid buffer solution (pH 4.61) to control acidity; the reducing sugars were determined by the Quisumbing and Thomas copper reduction procedure by direct weighing of cuprous oxide and also by the volumetric thiosulphate method on the same precipitates, recommended by the A.A.C.C.

A statistical analysis of the data showed that while the spread in the diastatic activities of the flours was lower for the improved method, this is more than counteracted by the much lower experimental error, with the result that this procedure gives a much better differentiation between the diastatic activity of different flours than either the gravimetric or volumetric copper reduction procedures. A difference of 7.5 units between the means of duplicate determinations on two flours was statistically significant for the ferricyanide procedure, while differences of 14.8 and 15.8 units would be required in the instance of the gravimetric and volumetric copper reduction procedures respectively.

For the entire series the Blish and Sandstedt method gave a significantly lower mean diastatic activity by 4.7 and 4.0 units, respec-

tively, than the gravimetric and volumetric copper procedures, due to the method giving appreciably lower values in the instance of flours of high diastatic activity. However, the correlations between the results by all methods were of a very high order of magnitude, and diastatic activity by the Rumsey method (Gravimetric determination of reduced copper) may be estimated from the Blish and Sandstedt values with a standard error, for a single predicted value, of $\pm$ 12.7 units. The Blish and Sandstedt improved method is to be preferred to the modified Rumsey method employing either the gravimetric or volumetric procedures for the determination of reduced copper from the standpoints of greater convenience, lower experimental error, and better differentiation between flours.

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THE VITAMIN B (B_1) AND G (B_2) CONTENT OF WHEAT PRODUCTS

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A few years ago a feeding experiment (Morgan and Barry, 1930) with school children carried on from this laboratory indicated the existence of appetite-stimulating and growth-promoting properties in wheat germ. No extensive examination of the actual vitamin value of the germ was made at that time but it seemed worth while later to continue the inquiry by animal assay of the product. Particularly it was desired to make a comparison of the vitamin content of the wheat germ with that of the whole wheat from which it was made and of the product resulting from a combination of the two. Certain claims of increase in vitamin B value of whole cereals resulting from the addition of small amounts of germ appear to be based on calculations from the supposed germ content of the grain rather than actual assays. This report describes the results of such actual assays.

Munsell and DeVaney (1933) have reported a careful study of the vitamins B and G of four samples of wheat germ among which they found B values ranging from 4.5 to 10.0 Sherman units per gram. Whole soft winter wheat they found to contain 1.5 units per gram. The vitamin G values in both cases were lower, ranging from 1.8 to 3.8 Sherman units per gram of germ and 0.8 units per gram of wheat. Hunt (1928), Aykroyd and Roscoe (1929), and Sherman and Axtmayer (1927) have published data on whole wheat which may be interpreted as signifying similar values. In no case have the germ and whole grain of the same origin been studied together by modern methods.

Methods

Both rats and pigeons were used in making the vitamin B (B_1) assays. The method of Chase and Sherman (1931) somewhat modified was used with the rats and that of Salmon (1927) with the pigeons. The other known nutritive needs of the test animals were met in both cases by the basal diet so that the only limiting factor was that of the vitamin B content of the products fed. The basal diet consisted of

extracted casein 18, Osborne and Mendel salt mixture 4, butterfat, 8, codliver oil 2, autoclaved yeast 10 or 15, cornstarch 53. Small accurately weighed doses of the test products were fed daily separately from the basal diet and the resulting weight increases of the young rats observed over a period of eight weeks. Weight maintenance and prevention of polyneuritis for four weeks was the criterion used in the case of the pigeons. Comparison was made in each case with results in comparable animals of the administration of known vitamin doses such as the International vitamin B standard, an acid clay adsorbate made from rice polish and distributed in 1932 by the vitamin standards committee of the League of Nations. A potent rice polish concentrate made according to the method suggested by Evans and Lepkovsky (1931) was also used in these comparisons and was the source of vitamin B (B_1) used with the vitamin G-free basal diet in the vitamin G (B_2) tests. Autoclaved yeast as 10 or 15% of the basal diet furnished the vitamin G (B_2) in the B-free diets.

The results of these studies are shown in four tables. Table I in-

TABLE I

VITAMIN B (B_1) CONTENT OF WHEAT PRODUCTS BY RAT GROWTH METHOD

Product	Daily dose	Number of rats	Average initial (Depleted weight)	Average gain in 8 weeks	International units per 100 g.
	Grams		*Grams*	*Grams*	
Whole wheat	0.40	4	73	−5	
	0.50	4	64	21	
	0.60	4	79	20	
	0.70	4	59	34	
	0.80	5	76	35	124
Wheat germ No. 1	0.07	12	75	6	
	0.10	12	66	24	
	0.15	5	70	39	725
Wheat germ No. 2	0.15	4	50	48	940
Wheat germ No. 3 (Bemax)	0.15	6	73	43	860
Whole wheat, with 10% added wheat germ	0.45	6	64	24	
	0.50	4	75	37	187
Whole wheat, with 20% added wheat germ (Wheat hearts)	0.20	7	68	15	
	0.30	6	79	18	
	0.40	4	64	30	220
Dry brewery yeast (Northwestern)	0.10	6	66	53	
	0.20	6	60	92	
	0.50	5	70	148	1260
International standard	0.01	26	68	34±2	10,000

dicates the antineuritic (vitamin B_1) value of several wheat germ and wheat samples as found by the rat growth method. The whole wheat, No. 1, was not as rich in this property as several other samples studied in this laboratory and elsewhere. The wheat germ designated as No. 1 [1] and made from this whole wheat was also slightly less potent than the other two samples examined. Sample No. 3, received from the distributors of Bemax, is apparently largely sterilized wheat germ although the manufacturers describe Bemax as "stablized entire cereal embryo." Wheat germ No. 1 was obtained in two lots designated by the millers as sterilized and unsterilized. These were tested separately with identical results and were therefore reported together. The same whole wheat reinforced with 10 and 20% additions of the wheat germ were also tested and found to contain progressively larger amounts of vitamin B_1, in almost the proportion calculable from the data on the whole wheat and wheat germ.

Similar relations were found by pigeon maintenance experiments as shown in Table II. Very nearly the same relative antineuritic values were found in these four wheat products by this as by the rat growth method. The pigeon maintenance dose was apparently 5 to 6 times the rat growth unit. When compared on the four products, whole wheat, wheat germ, whole wheat with 10 and with 20% germ added, the ratio of pigeon (International) unit to rat unit is 5.5, 5.9, 7.1, and 5.0. Calculated according to the formulae of Cowgill and co-workers (1934) the ratio for animals of the weights used in these experiments should be about 4.5.

Under the conditions of these tests our young rats, average weight 85 g., seemed to require nearly the same amount of vitamin B per gram of body weight as did our mature pigeons, 400 to 450 g. weight.

The pigeon maintenance value of the International standard clay adsorbate reported by Cowgill (1934) is about twice the value which we found by our pigeon technique. The difference is obviously due to the difference in method of assay. We force fed the birds daily with a constant amount of the basal diet and used a period of 28 days for each dose of the test material fed. Cowgill administered only part of the basal diet by hand allowing the birds to eat polished rice *ad libitum* and used maintenance of body weight for a minimum of one week as criterion of protection. Our technique represents a more severe test of antineuritic property of the material examined but is at the same time probably a safer and more delicate means of comparing similar products.

Our experience with the growth of young rats on the International standard as sole source of vitamin B is apparently somewhat different

[1] The wheat, wheat germ, and mixtures of the two were prepared for us by the Sperry Flour Company, San Francisco.

from that of others. On our B-free basal diet rats weighing 50 to 80 g. and depleted of vitamin B reserves gain an average of 34 g. in eight weeks when given daily 10 mg. of the standard preparation. This performance has been used as representing the effect of 1 International

TABLE II

VITAMIN B (B_1) CONTENT OF WHEAT PRODUCTS BY PIGEON METHOD

Pigeon number	Average weight	Product	Dose	Period of maintenance	International units per 100 g.
	Grams		*Grams*	*Days*	
5	430	Whole wheat	5.90	28	
	432	" "	5.30	28	
	430	" "	4.80	28	
	440	" "	4.30	28	
	440	" "	3.87	7	
7	425	Whole wheat	5.80	28	
	425	" "	5.22	28	
	425	" "	4.70	5	126
6	415	Wheat germ, unsterilized	0.90	28	
	430	" " "	0.80	28	
	430	" " "	0.72	28	
8	460	Wheat germ, unsterilized	0.80	42	
	460	" " "	0.72	28	
9	445	Wheat germ, sterilized	0.90	28	
	450	" " "	0.85	56	
	445	" " "	0.79	14	
10	430	Wheat germ, sterilized	0.70	28	
	440	" " "	0.63	28	
12	430	Wheat germ, sterilized	0.80	28	792 (average for wheat germ)
8	450	Whole wheat with 10% added wheat germ	3.60	37	
	440	" " " " " " "	3.30	15	
11	360	Whole wheat with 10% added wheat germ	4.00	28	
	360	" " " " " " "	3.60	18	180
10	408	Whole wheat with 20% added wheat germ	2.25	28	
			2.03	14	
12	435	Whole wheat with 20% added wheat germ	2.80	28	
	430	" " " " " " "	2.52	28	
	430	" " " " " " "	2.27	28	
	430	" " " " " " "	2.04	9	264
26	430	International standard	0.07	28	
10	408	" "	0.05	28	
2	402	" "	0.05	28	10,000

unit, and the number of units per 100 g. of test material is calculated from the formula:

$$U = \frac{W \times 100}{34 \times D},$$

in which U is the number of units per 100 g., W the total average gain in grams of the rats in eight weeks on D, the weight in grams of the daily dose of test material. Since the Chase and Sherman unit is based on a similar growth of 25 g. in eight weeks our International unit is equivalent to 1.36 Chase and Sherman units of vitamin B.

Since 60 mg. of the standard adsorbate are needed for protection of pigeons of the size used, 350 to 450 g., the number of units per 100 g. of test material may be estimated by determining the minimum daily dose which yields the same amount of protection as 60 mg. of the standard (6 units) and calculating from the formula: $(100 \times 6/D)$ in which D is the daily minimum dose of the test material.

It is obvious that calculations of the probable vitamin content of germ-reinforced cereals based on the assumption that nearly all the vitamin B of the wheat is located in the germ and that the maximum weight of germ in the wheat kernel is 2% may be far from correct. In such assumptions the wheat containing 10% added embryo should have about 750 units of vitamin B per 100 g. and that containing 20% added embryo about 1350 units. But the actual amounts are 184 and 242, respectively. The wheat germ used in these products is also apparently nearly as potent as any germ preparations available or hitherto reported.

The agreement in our present series between rat growth and pigeon maintenance tests in terms of International units per 100 g. is excellent.

The Vitamin G (B_2) Results

The vitamin G (B_2) content of these foods was found to be relatively less than their B_1 contents. This is in confirmation of several earlier reports by Hunt (1928), Sherman and Axtmayer (1927), Hetler, Meyer, and Husseman (1931), and Munsell and DeVaney (1933). In Table III are shown the details of these experiments as well as findings with several dried concentrates reputed to be rich in vitamin G. It is interesting to note that the concentration of vitamin G in the germ is less marked than is the case with vitamin B. Thus the proportion of the former in wheat germ to whole wheat is 6:1 but of the latter is 4:1. The whole wheat reinforced wtih 20% germ as compared with whole wheat has 1.9:1 and 1.2:1, respectively, of vitamins B and G. Some discrepancy occurred in the G content of the whole wheat and 20% wheat germ mixture since its assayed value was only 24% higher than that of the wheat alone. The calculated value is at least 60% higher.

Apparently vitamin G is distributed in the endosperm more generously than is vitamin B. This may help to account for Osborne and Mendel's (1919) finding that the endosperm contained more "water-soluble vitamin" than did the embryo.

TABLE III

VITAMIN G (B_2) CONTENT OF WHEAT PRODUCTS AND CERTAIN DRIED FOODS

Product	Daily dose	Number of rats	Initial (depleted) weight	Gain in 8 weeks	Units per 100 g.
	Grams		*Grams*	*Grams*	
Whole wheat	1.62	4	60	54	
	2.00	8	83	53	
	3.25	4	61	65	100
Whole wheat with 20% wheat germ added	0.87	4	64	26	
	1.50	4	60	48	124
Wheat germ	0.25	4	53	32	
	0.50	15	65	47	400
Whole egg (dried)	0.50	8	68	56	448
Whole milk (dried)	0.25	8	61	35	488
	0.50	7	77	52	
Beef liver (dried)	0.12	4	69	47	
	0.25	18	59	99	1580
Brewery yeast (dried)	0.12	2	74	61	
	0.25	8	67	88	1593
Controls	0.0	21	58	5	

If all the vitamin B were concentrated in the germ then the sample which we tested would represent 1.7% of the wheat from which it was made. But on the basis of a similar concentration of vitamin G the germ would represent 2.4% of the wheat. Milled wheat products such as farina and white flour may therefore be expected to carry more of the vitamin G of the whole wheat than of its vitamin B.

The dry solids of eggs and of milk have only a little more vitamin G than does wheat germ, but nearly five times as much as whole wheat. However, on the basis of fresh weight the wheat germ has 3.55 units per gram, whole eggs 1.16, whole milk 0.63, whole wheat 0.88. Dried beef liver and dry brewery yeast appear to be nearly the same in vitamin G value having about four times the amount present in this wheat germ, dried eggs, and dried milk. In the fresh state this liver had 4.58 units of vitamin G per gram.

The vitamin G unit used is defined as that amount of vitamin which

will induce a gain in weight of 25 g. during eight weeks in young rats depleted of vitamin G reserves and fed a basal diet adequate in all other known diet factors with the exception of vitamin G. The basal diet was similar to that used in vitamin B (B_1) tests except that the autoclaved yeast was omitted and a rice polish concentrate fed separately as source of B. The animals fed this diet but without source of vitamin G grew very little but usually did not die or lose much weight. They usually exhibited lesions of the forepaws, ears, and eyelids, loss of fur, extreme nervousness and in some cases well defined bilateral cataracts.

In Table IV is presented a summary of both vitamin B and G findings, an average of rat and pigeon values being used in the former when possible.

TABLE IV

SUMMARY OF VITAMIN B (B_1) AND G (B_2) CONTENT OF WHEAT PRODUCTS

Product	Units of vitamin B (B_1) per 100 g.		Units of vitamin G (B_2) per 100 g.
	International	Sherman	
Whole wheat No. 1	125	170	100
" " No. 2	189	257	
" " No. 3	142	193	
Wheat germ, No. 1	758	1032	404
" " No. 2	940	1278	
" " No. 3	860	1170	
Whole wheat No. 1 with 10% added germ No. 1	184	250	
Whole wheat No. 1 with 20% added germ No. 1 (Wheat hearts)	242	329	124
Dry brewery yeast	1260	1714	1593
Whole wheat bread	186	253	
	167	227	

Summary

The International unit of vitamin B (10 mg.) provides 34 g. gain in 65 to 70 g. rats during eight weeks and is therefore equivalent to 1.36 Chase and Sherman units. For maintenance of weight over four weeks and prevention of polyneuritis in pigeons of 350 to 450 g. weight about 60 mg. or 6 units of the International standard preparation is required. Close agreement can be expected between values obtained by these 2 methods.

Wheat germ (Sperry) separated from a given wheat sample contains six times as much vitamin B and four times as much vitamin G as did the whole wheat.

A mixture of the whole wheat and 10% added germ contained nearly 50% more B than did the whole wheat, and a similar mixture of wheat and 20% added germ contained nearly twice as much B and 24% more G than the whole wheat.

The solids of cow's whole milk, whole eggs, and wheat germ carry nearly the same amounts of vitamin G. Dry brewery yeast and dried beef liver have about four times as much as wheat germ. In the fresh state however wheat germ has three times as much G as eggs, five to six times as much as cow's milk, and about two-thirds as much as beef liver.

The vitamin G of wheat is less concentrated in the germ than is the vitamin B.

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MINUTES OF THE TWENTY-FIRST ANNUAL MEETING OF THE AMERICAN ASSOCIATION OF CEREAL CHEMISTS

Brown Palace Hotel, Denver, Colorado

June 4–8, 1935

M. D. Mize, *Secretary-Treasurer*

On Monday evening, June 3, at 8:15 p.m. an illustrated lecture was given in the Grand Ballroom of the Brown Palace Hotel, by Dr. F. M. Van Tuyl, Professor of Geology, Colorado School of Mines, Golden, Colorado, on "The Geology of the Colorado Rockies."

Tuesday, June 4

Meeting called to order at 10:00 a.m. by President Mary M. Brooke.

Invocation by Rev. A. W. Liggitt.

Due to the tragic flood conditions in several parts of Colorado, the Honorable Ed. C. Johnson, Governor of the State of Colorado, found it impossible to be present and in his stead F. H. McInroy, Commissioner of State Budget Control, delivered a message of welcome to the City of Denver and the State of Colorado.

Messages of greeting were received from J. A. Dunn, A. E. Cliffe, Rowland J. Clark, C. E. Mangels, members of the Association who found it impossible to be present. Messages of greeting were also read from the Association of Operative Millers, Cincinnati Hotel Association, Greater National Capital Committee, Russell Wilson, Mayor of Cincinnati, and Cincinnati Chamber of Commerce.

Harry D. Liggitt, Chairman of Local Arrangements Committee, was presented before the convention and extended a cordial welcome to all members present, outlining the general activities of the week and making several special announcements regarding such arrangements.

In reverence to the memory of T. A. Gierahn, G. E. Howe, and M. B. Graff, members of the Association who had passed away since our twentieth annual meeting, Washington Platt read an obituary and all members present stood in silent reverence.

President Mary M. Brooke delivered the address given on pages 436–440 of this issue of CEREAL CHEMISTRY.

James T. Jardine, Chief, Office of Experiment Stations, United States Department of Agriculture, Washington, D. C., delivered an address "Cereal Work of the Agricultural Experiment Stations."

The following committees were appointed by President Mary M. Brooke:

Nominating Committee: L. D. Whiting, Chairman, G. E. Findley, C. B. Kress.

Resolutions Committee: C. B. Morison, Chairman, R. C. Sherwood, Perie Rumold.

C. H. Bailey moved that messages of greeting and best wishes be sent to C. E. Mangels and A. E. Cliffe, who were prevented from attending the convention due to serious illness. Seconded; carried.

Dr. F. L. Dunlap was presented before the meeting and a standing vote of thanks was given him for his work in arranging the Twenty-First Annual Meeting Program.

Meeting adjourned at 12:15 p.m. by President Mary M. Brooke.

Wednesday, June 5

Meeting called to order at 9:00 a.m. by President Mary M. Brooke, and placed in charge of C. B. Morison, Chairman of the morning session.

Paper—"Influence of Various Factors, Including Altitude, in the Production of Angel Food Cakes," by M. A. Barmore, Colorado State Experiment Station.

Paper—"Physical Differentiation of Flour Types," by M. C. Markley.

Paper—"Ash of Flour in Self-Rising and Phosphated Flours," by R. A. Barackman and E. A. Vaupel, read by R. A. Barackman.

Paper—"Studies in Toxic Wheat Grown on Soils Containing Selenium," by D. B. Jones, Bureau of Chemistry, Washington, D. C.

Paper—"Free and Bound Water in Doughs," by O. Skovholt and C. H. Bailey, read by O. Skovholt.

Paper—"Cereals in Nutrition Today," by F. L. Gunderson.

Paper—"Experimental Durum Milling and Macaroni Making Technique," by D. S. Binnington and W. F. Geddes, read by W. F. Geddes.

Meeting adjourned at 12:00 noon.

Thursday, June 6

At 7:30 a.m. a breakfast and meeting was held for the Officers of the Association and the Chairman of the Local Sections.

Meeting called to order at 8:30 a.m. by President Mary M. Brooke and placed in charge of R. C. Sherwood, Chairman of the morning session.

Paper—"Problems of the Baker for the Cereal Chemists," by G. H. Darnell, Past President of the American Society of Bakery Engineers.

Report of Committee on Methods of Analysis by Betty Sullivan, Chairman.

Sub-report—"Forced Draft as an Aid in Moisture Tests," by C. F. Davis.

Sub-report—"Gassing Power of Flour," by R. M. Sandstedt, read by M. J. Blish.

Sub-report—"A Collaborative Study of Moisture Methods for Mill Feeds," by A. Treloar and Betty Sullivan, read by Betty Sullivan.

Sub-report—"A Study of Yeast Variability as Measured by the Fermentograph," by Cleo Near and Betty Sullivan, read by Betty Sullivan.

Sub-report—"The Use of the Farinograph as an Accurate Measurement of Absorption," by Cleo Near and Betty Sullivan, read by Betty Sullivan.

Sub-report—"Viscosity," by E. G. Bayfield, read by W. E. Brownlee.

Sub-report—"The Book of Methods," by C. E. Mangels, read by Betty Sullivan.

Paper—"A Comparative Study of Bread Leavened with Yeast and with Hydrogen Peroxide," by L. H. Bailey and J. A. LeClerc, read by Charlotte Chatfield.

Paper—"White Wheat Variety Studies," by C. C. Fifield, S. R. Snider, R. Weaver, and H. Stevens, read by C. C. Fifield.

Paper—"The Enigma of Starch," by C. H. Bailey.

President Mary M. Brooke resumed charge of the meeting to conduct a business session and election of officers.

Messages of greeting were received and read from W. A. Richards, Sam Lawellin, and C. W. Brabender, members; also from Morten Milling Company and the Kimbell Diamond Milling Company of Texas.

Washington Platt was recognized by the Chair and presented the following:

"The Executive Committee has studied the question of separating the office of Secretary-Treasurer into two individual offices and in order to state exactly what we have in mind and exactly what our conclusions are, I will take the liberty of reading the following:

"Our Association is most fortunate in its remarkable growth. Not only has the membership doubled and trebled, but the number of local sections has constantly increased in number and in the importance of their work. The same thing may be said of our scientific committees.

"Growth of the Association has brought about a necessary increase in expenditures. The Association has always pursued a policy of economy and conservation. We have always lived within our income, and there has never been a disposition to spend good money on useless frills just because we had it to spend. We have no idea whatever of departing from our historic policy of economy and conservation.

"Growth has brought its own problems. Since the formation of the Association, the duties of Secretary-Treasurer have been performed by one man. The Association has been most fortunate in enjoying the faithful and efficient services of R. K. Durham and M. D. Mize. The burden of carrying on the duties of the two positions, namely, Secretary and Treasurer, has become increasingly great and we feel that no one person should be burdened with the duties of both offices for the best interests of the Association.

"After a careful study of the situation which has been brought about by the growth of the Association, the Executive Committee recommends the following program to the Association:

"First, the Constitution shall be amended so as to provide for a Secretary and a Treasurer. For this purpose constitutional amendments will be submitted for the action of this meeting in a few minutes.

"Second, the duties of the Secretary are defined in the constitutional amendment as follows:

"'Article IV, Section 4(*c*), The Secretary shall keep a record of the minutes of the general meetings and carry on the general correspondence of the Association. He shall also maintain close contact with the local sections, and carry on intersectional correspondence.'

"In addition to these constitutional duties it is hoped that the Secretary will be able to relieve the President's office of a part of the enormous body of necessary work which has necessarily grown up in coordinating the activities of the Association.

"The third step in the program is economy. The Executive Committee will limit the expenditures of the Secretary's office, so that the combined expenses of the Secretary's office and the Treasurer's office under the new plan will be no greater than it would have been with normal increase of expenses under the old plan where all of the work was carried out under the supervision of one officer. The Executive Committee will establish a budget for each office; and each officer will be required to live within it. This will prevent unnecessary expense and elaboration of duties.

"The Executive Committee believes that this plan will meet this problem which has arisen from the growth of our Association and that this plan will prevent an unnecessary burden being placed on the shoulders of any one man. The Committee is convinced that the program will make for efficiency and economy.

"The Executive Committee lays this program before the meeting for discussion and unanimously recommends its adoption."

Washington Platt moved that Article IV, Section 1 of the Constitution, be changed to read "The elective officers of this Association shall be six in number, President, Vice-President, Secretary, Treasurer, Editor-in-Chief of CEREAL CHEMISTRY, and Managing Editor." Seconded by C. H. Bailey; carried.

Washington Platt moved that Article IV, Section 4(*c*) of the Constitution, be changed to read "The Secretary shall keep a record of the minutes of the general meetings and carry on the general correspondence of the Association. He shall also maintain close contact with the local sections and carry on inter-sectional correspondence." Seconded, by G. E. Findley; carried.

Washington Platt moved that Article IV, Section 4(*d*) of the Constitution, be changed to read "The Treasurer shall collect all fees and moneys due the Association and pay all bills by check and shall record all such receipts and expenditures. Bills shall not be paid except with the approval of the Vice-President and all checks issued shall be countersigned by the Vice-President. The Treasurer shall also keep the membership roster of the Association." Seconded, by L. W. Francis; carried.

Report of the Nominating Committee

L. D. Whiting, *Chairman*

Nominated for President, Washington Platt.
Nominated for Vice-President, C. H. Bailey, W. F. Geddes, H. D. Liggitt.
Nominated for Secretary, W. R. Green, H. W. Putnam, H. G. Walter.
Nominated for Treasurer, M. D. Mize.
Nominated for Editor-in-Chief of Cereal Chemistry, D. A. Coleman.
Nominated for Managing Editor, C. C. Fifield.

Elsie Shover moved that the report be accepted. Seconded; carried.

L. D. Whiting moved that when three or more candidates are nominated for any one office, the lowest candidate on each ballot be dropped and another ballot taken on the remaining candidates, until one candidate obtains the majority of all votes cast. Seconded, by C. N. Frey; carried.

Election of President.

M. A. Gray nominated H. D. Liggitt for President; seconded, by Wm. R. Green.

R. B. Potts moved that the nominations be closed. Seconded, by E. E. Smith; carried.

Washington Platt was elected President.

Election of Vice-President.

F. A. Colatz moved that the nominations be closed. Seconded, by M. J. Blish; carried.

H. D. Liggitt was elected Vice-President.

R. C. Sherwood moved that the election of Treasurer, Editor-in-Chief, and Managing Editor proceed at this time. Seconded; carried.

M. J. Blish moved that the rules be suspended and the Secretary-Treasurer be instructed to cast an unanimous ballot for M. D. Mize as Treasurer, D. A. Coleman as Editor-in-Chief, and C. C. Fifield as Managing Editor. Seconded, by P. P. Merritt; carried.

Election of Secretary.

Pearl Brown moved that the nominations be closed. Seconded, by C. B. Morison; carried.

H. W. Putnam was elected Secretary.

Meeting adjourned at 1:00 p.m.

Friday, June 7

Meeting called to order by President Mary M. Brooke and placed in charge of W. F. Geddes, Chairman of this Session for the morning.

Report of Committee on Standardization of Laboratory Baking, W. F. Geddes, Chairman, who gave a general report of the work undertaken during the year.

Sub-report—"A Cooperative Study of the Utility of Different Methods for Evaluating Flour Strength," by D. W. Kent-Jones and W. F. Geddes, read by W. F. Geddes.

Sub-report—"Experimental Baking Pans. Report of the Pioneer Section Research Committee," by C. F. Davis.

Sub-report—"Supplementary Tests for Measuring Flour Strength," by M. J. Blish.

Sub-report—"Correlation of Experimental and Commercial Baking Tests," by J. Freilich, S. McHugh, and C. N. Frey, read by J. Freilich.

Paper—"Variation in Mineral Content of Cereals," by Charlotte Chatfield, Bureau of Home Economics, Department of Agriculture, Washington, D. C.

Sub-report of Committee on Standardization of Laboratory Baking—"Studies on Test Dough Mixer Calibration," by Quick Landis and J. Freilich, read by Quick Landis.

Sub-report—"Study of the Regular Hobart-Swanson Mixer and Compared with the Hobart Mixer with Two Dough Hooks and the Cake Paddle for Mixing Bread Doughs," by D. A. Coleman, R. Weaver, and C. Bode, read by C. C. Fifield.

Sub-report—"A Micro Experimental Flour Mill," by W. F. Geddes and B. Frisell, read by W. F. Geddes.

Sub-report—"The Utility of a Micro Milling and Baking Technique in Evaluating Wheat Strength," by W. F. Geddes and T. R. Aitken, read by W. F. Geddes.

Sub-report—"A Further Study of the Determination of Flour Absorption by Means of the Supercentrifuge," by E. B. Working.

Sub-report—"The Effect on Gas Production of Substituting Various Salts for Sodium Chloride in the Baking Formula," by R. K. Larmour and H. N. Bergsteinnson, read by W. F. Geddes.

Report of the Committee on Cake Baking Tests, G. L. Alexander, Chairman.

Sub-report—"Photography as a Means of Obtaining Permanent Records for Cake Baking Tests," by J. R. Davies.

Sub-report—"Sugar and Shortening Tolerance of a Short Patent and a Fancy Clear Flour," by J. R. Davies.

Sub-report—"Suggested Tentative Specifications for the Dried Egg Albumin Used in the Basic Cake Baking Test," by C. A. Glabau.

Sub-report—"Results Secured by the Basic Cake Baking Method when Using Three and Ten Quart Bowls, and Beaters and Whips in the Mixing Operation," by H. R. Fisher.

Sub-report—"Determining Sugar Tolerance in Supplement A by Adding the Sugar in Solution instead of in the Dry Form," by W. E. Stokes.

G. L. Alexander, Chairman of the Committee, then gave a general report.

Report of the Committee on Testing Rye Flour, by L. H. Bailey, Chairman.

Session adjourned at 12:00 noon.

Session of the Malt and Barley program called to order at 8:15 a.m. by D. A. Coleman, Chairman.

Report of the Committee on Standardization of Methods for Brewing and Malting Control, D. A. Coleman, Chairman, who gave a general report of the work undertaken during the year.

Paper—"Recent Developments in European Brewing Chemistry," by Elsie Singruen.

Paper—"Development of New Malting Barleys and their Properties," by J. G. Dickson, read by D. A. Coleman.

Paper—"The Preparation and Commercial Uses of Malt Products," by G. S. Bratton.

Paper—"Malting and Mashing," by H. W. Rohde, read by Elsie Singruen.

Paper—"Determination of Moisture in Barley Malt; a Comparison of Methods and Apparatus for this Purpose," by D. A. Coleman and S. R. Snider, read by D. A. Coleman.

Session adjourned at 12:00 noon.

Meeting called to order at 12:00 noon, by President Mary M. Brooke.

Paper—"Yeast," by C. N. Frey. This paper was accompanied by a motion picture on the "Manufacture of Yeast," supplied by Standard Brands, Inc.

C. B. Morison moved that a rising vote of thanks be extended to C. N. Frey and Standard Brands, Inc., for their generous cooperation in presenting this paper and picture before our convention. Seconded; carried.

Meeting adjourned at 1:00 p.m.

Meeting called to order at 2:30 p.m. by W. F. Geddes.

Report of the Committee on Methods of Testing Self-Rising Flour, by H. G. Walter, Chairman.

Report of the Committee on Testing Biscuit and Cracker Flour, by R. M. Bohn, Chairman, read by H. J. Loving.

Report of the Committee on Testing Pie Flour, by C. B. Kress, Chairman.

President Mary M. Brooke resumed charge of the meeting.

W. F. Geddes moved that the following recommendations of the Committee on Standardization of Laboratory Baking be accepted:

"That a comprehensive study of bakery tins, made of 2XX tin, be undertaken on the influence of size and shape of pan and pan treatment, such as greasing and darkening from the standpoint of the variability between replicates and differentiation between flours. Since the tall form tin is in general use, it should be employed as a standard reference.

"In view of the fact that members are experiencing difficulty in securing the Hobart-Swanson Mixer, the Association should take this matter up with the Hobart Manufacturing Company.

"That a further study be made of the method of reporting the results of the Standard Baking Test."

Seconded, by C. B. Morison; carried.

D. A. Coleman moved that the following recommendations of the Committee on Standardization of Methods for Brewing and Malting Control be accepted:

First, That the work of the Committee be continued, and,

Second, That the Methods of the Malt Analysis Standardization Committee of the Brewer-Maltster-Scientific Station Association be adopted by the A.A.C.C. as tentative methods for study.

It is further recommended that studies with respect to the analysis of malt adjuncts be undertaken.

Seconded, by F. A. Collatz; carried.

H. G. Walter moved that the following recommendations of the Committee on Methods of Testing Self-Rising Flours be accepted:

"That the collaborative baking work be continued along the lines followed by the committee during the past year, in order that we further test the reliability of our baking tests."

Seconded, by F. A. Collatz; carried.

H. J. Loving moved that the following recommendations of the Committee on Testing Biscuit and Cracker Flours be accepted:

"Compare flours of same viscosity but widely varying protein, chiefly in relation to commercial utility.

"Continue work with clears and short patents to see whether viscosity classifies them correctly.

"Effect of bleaching in changing character of flour as found in bakeshop compared to change in viscosity and laboratory baking tests."

Seconded, by W. E. Brownlee; carried.

G. L. Alexander moved that the following recommendations of the Committee on Cake Baking Tests be accepted:

"The report by W. E. Stokes indicates that Supplement A is best conducted by adding the sugar increments in dry form instead of in solution.

"The Committee recommends the use of photographs, similar to those shown and described by J. R. Davies, in conjunction with the Official Cake Score; to record and preserve records of cake baking tests. It is also suggested that a study be made, leading to the preparation of type photographs, to be used as an aid in classifying external and internal characteristics of test cakes. This study would be parallel to the study conducted some years ago for a similar purpose in the case of "pup" loaves.

"H. R. Fisher has shown, by his study of the mixing procedure, that the results obtained through the use of varying sizes of bowls, and by beaters and whips, will vary. Therefore, beaters and whips are not interchangeable; and the bowl size and beater specified in the Official Cake Baking Method must be adhered to for standard results.

"The work by Mr. Fisher was conducted at a high altitude, in Idaho; and indicates the need for preparing a chart which will show the necessary adjustment of the quantity of soda and cream of tartar for different atmospheric pressures. This chart would be for the use of laboratories working in the higher altitudes.

"The specifications suggested by C. A. Glabau should be adhered to when securing dried egg albumin for use in the Official Cake Baking Test. The Committee recommends that intensive collaborative work should be conducted next year in order to finish as soon as possible the study of albumin. This appears to be the most important single problem now faced by the Committee. Also, in view of the fact that many technicians have had difficulty in making satisfactory test cakes with dried albumin, the Committee suggests the alternative use of fresh or frozen egg albumin in the Official Formula; provided that the type of albumin be described in connection with the score."

Seconded, by G. E. Findley; carried.

C. B. Kress moved that the following recommendations of the Committee on Testing Pie Flour be accepted:

"Continue the Pie Flour Collaborative Tests.

"Further work on filled pies to arrive at a generally satisfactory standard pie filling that will incorporate all the advantages of the citric acid filling and the peach filling."

Seconded, by E. E. Smith; carried.

Betty Sullivan moved that the following recommendations of the Committee on Methods of Analysis be accepted:

"That the methods for the measurement of the gassing power of flour be given further study.

"That the vacuum oven and air oven (130° C. for 1 hour) methods be continued as the official moisture methods for wheat feeds as well as flour.

"It is respectfully suggested to the incoming President that hereafter members of the Methods Committee be so chosen that each member will be responsible for collaborative work and a review of new developments in a particular field, such as proteins, diastatic activity, and gassing power, testing of shortenings, leavening agents, etc.

"Such an allocation of the work would be very helpful if another revision of 'Cereal Laboratory Methods' is undertaken in the future, and would serve as well to inform cereal chemists of applicable or promising new methods both for control and research work."

Seconded, by O. Skovholt; carried.

W. F. Geddes moved that the following recommendations of the Committee on Testing Rye Flour be accepted:

"The work be continued.

"The next committee study a greater number of samples of the same grade of rye.

"Ash be determined on the rye flours.

"The relation of ash contents of rye flour to the color, odor, and taste of the rye bread be studied.

"Consideration be given as to proofing periods most suitable for the different grades of rye.

"The values for the different items in the score card be given further consideration."

Seconded, by Elise Shover; carried.

L. W. Francis moved that the Minutes of the 1934 Convention by M. D. Mize, Secretary-Treasurer, be approved as printed in CEREAL CHEMISTRY, Vol. 11, No. 4, pages 431–445, and further that the Annual Financial Report of the Secretary-Treasurer for the year 1934 be approved as printed in CEREAL CHEMISTRY, Vol. 12, No. 2, pages 172–174. Seconded, by R. B. Potts; carried.

H. H. Johnson moved that the Auditing Committee Report be approved as printed in CEREAL CHEMISTRY, Vol. 12, No. 2, page 174. Seconded, by M. A. Gray; carried.

Report of the Executive Committee

Washington Platt, *Chairman*

It is the primary duty of the Executive Committee to carry on the business activities of our Association. The purposes of our Association, as expressed in our Constitution, are scientific, so that the business affairs must be regarded as *necessary evils*. In calling them so, I would emphasize both words.

No one will deny that business affairs are necessary. Without membership, dues, money in the bank, etc., most of the purely scientific activities of our Association would be impossible. Without subscriptions, pay rolls, etc., our very high class journal could not be published. The A.A.C.C. is to be congratulated in that it has always been on a sound basis financially, and has lived within its income. We regard it as distinctly worth mentioning that it is the policy of this Association that each major activity should pay its own way. For example, our journal, CEREAL CHEMISTRY, pays for itself, and meeting expenses are paid out of meeting fees. All this has been a great help to our scientific and social activities.

Now for the evil. There is a definite tendency for the mere machinery of running the Association to become so elaborate as to be unnecessarily burdensome. It also tends to become expensive. It would be a great pity if the routine duties of the officers should become so heavy that many good men would find it impossible to assume them, or if our meetings should become so elaborate as to overwhelm the local arrangements committee and require an ever larger Convention fee. In our opinion we should now be on our guard against increasing the amount of machinery necessary to run the A.A.C.C.

As in former years, the Executive Committee has had general supervision of all the financial affairs of the Association. In the present year this has included not only the regular business of the Association, and the publication of CEREAL CHEMISTRY, but also the special activities which have so greatly distinguished the past twelve months. These are: Decennial Index, the History, and Cereal Laboratory Methods. The credit for these achievements, of which we can all be so proud, belongs with those members of our Association who have had them in charge; but the blame, if their cost had exceeded our capacity to pay, would have rested with the Executive Committee.

With the growth in our Association and in the number of local sections, the choice of the meeting city each year has become more complicated. Two years ago Dr. Sherwood published in the News Letter a map which gave us all a clearer picture of the geographic distribution of our membership. This year the Executive Committee submitted a plan for the future choice of meeting cities. This plan contained two propositions. First, the city should be chosen more than one year in advance. Under the old plan the city was often not decided until six months or so before the meeting. This was a handicap to the Local Arrangements Committee, to the Program Committee, and to the Officers of the Association. Under the new plan we know at the current meeting where the next one is to be. Committees can be appointed and plans made while the members are together. Second, the continent was divided into three areas, Northwest, Southwest, and Eastern. It was provided that normally the meeting would be held in these areas in rotation. This was not made obligatory, so that this arrangement can be varied

for sufficient cause. The final decision rests, as before, with the Executive Committee. We expect this method to provide a properly balanced schedule of meetings throughout the years which will be fair to all. The plan was adopted by a vote of the membership as follows: Favorable 163; favorable with reservations 4; unfavorable 17; that is, the vote was nearly 10 to 1 in favor of the new plan.

After a very careful study the Committee decided unanimously in favor of Dallas for our 1936 meeting. This will be held in connection with the Texas Centennial celebration. We have the most splendid co-operation from the Lone Star Section. We are glad indeed to have such a favorable opportunity to meet in this very important section of the country.

History records that our Constitution has been frequently amended to keep it in step with changing conditions. This year the Executive Committee set itself the task of going over the whole Constitution and proposing those amendments which seemed necessary. Most of the amendments proposed constituted minor but self-evident improvements. Our present constitution has worked excellenlty and no radical changes were proposed. The most important change is that of separating the office of Secretary and Treasurer. These amendments have been published in the News Letter. They will be submitted to the membership with the recommendations of the Executive Committee for discussion and vote later during the present session.

The present Executive Committee has put the business of the Association on a somewhat more business-like basis, making a permanent record of what regular expenses are authorized and providing permanent minutes and permanent files of the more important material. This will facilitate the business of the Association, will avoid misunderstandings and should some day have considerable historic interest.

The Committee has approved the petition for separation of the Cincinnati Division from the Central States Section, and the granting of a charter to this group to be known as the Cincinnati Section.

The present Executive Committee has continued the policy of living within our means. Financially the past twelve months have been successful. Great credit is due to our members who have helped to bring this about. To the Local Arrangements and similar Committees who have so carefully lived within their budgets, to the editors of CEREAL CHEMISTRY and especially to our most efficient Secretary-Treasurer, we extend thanks for their excellent co-operation.

Washington Platt moved that this report be accepted. Seconded, by M. C. Markley; carried.

L. R. Olsen moved that Article IV, Section 3(*a*), be changed to read as follows: "The President at or preceding each general meeting, shall appoint a Nominating Committee, which committee shall present at least one name for each of the six elective offices of the Association." Seconded, by M. C. Markley; carried.

R. A. Barackman moved that Article IV, Section 4(*d*), be relettered to read Article IV, Section 4(*e*), and further that Article IV, Section 4(*e*), be relettered to read Article IV, Section 4(*f*). Seconded, by Paul Logue; carried.

Report of the History Committee

Rowland J. Clark, Chairman

The History Committee set out this year to accomplish just one objective and that was to write up the history of the Association from 1930 down to the present administration. This objective has been attained. It is with considerable relief that the Committee is submitting to the Association its version of the main events in the life of the Association for the past four administrations. It is recommended that these writings be passed on to the History Committee of next year for careful revision and criticism; thereby adding to and subtracting from the present paragraphs until through the labor of many minds the resulting record reflects the true and accurate cross section of Association activities.

In addition to writing up the history of the past four administrations, the Committee took over the Association scrap book which had been started by the Publicity Committee a few years ago and has brought the book up to date by pasting into it various notices and writings pertaining to the Association happenings during the year. Grateful acknowledgment is hereby given the Chairman of

the Publicity Committee, Mr. Putnam, for his splendid co-operation in forwarding the History Committee all Association press releases as they were given out for publication. The members of his Committee likewise co-operated to the fullest extent. It is believed, that by keeping this scrap book, the History Committee as well as any Association member may "see ourselves as others see us."

Lastly, but by no means least, the Committee co-operated with the previous History Committee in bringing into permanent form the History of the Association from the beginning in 1915 to 1930. The booklet just recently printed sets forth the untiring efforts of the previous History Committee. Too much praise cannot be given Mr. Ralph Mitchell, former Chairman of this Committee, for sticking with his job until it was so successfully accomplished. Much of the proof-reading drudgery fell to the lot of Ralph Mitchell, Leslie Olsen, R. C. Sherwood, and Sam Lawellin. Dr. Sherwood carried the responsibility of dealing with the printers.

It is therefore believed that in presenting this report to the Association, the History Committee has completed its work up to date.

L. R. Olsen moved that this report be accepted. Seconded, by H. G. Walter; carried.

L. D. Whiting moved that Article IV, Section 5(*c*), shall be changed to read as follows: "The Executive Committee shall co-operate with the President in carrying on the business of the Association between meetings. The Executive Committee shall have power to control the policy of the Association between meetings, including finances of all Association activities." Seconded, by P. E. Minton; carried.

Paul Logue moved that Article IV, Section 6, be changed to read as follows: "The President shall appoint three or more active members before each general meeting to act as an Auditing Committee. It shall be the duty of this committee to examine the books of the Treasurer and the books of CEREAL CEMISTRY. Other financial records of the Association may also be examined at the discretion of this Committee. The Auditing Committee shall report its findings to the Association. This Committee, with the approval of the Executive Committee, may employ a certified accountant to audit these books." Seconded, by R. A. Barackman; carried.

W. F. Geddes moved that Article V, Section 6, be changed to read as follows: "Assessments not to exceed one year's dues may be levied in any one year when the current expense of the Association makes this necessary. The Treasurer may recommend the levy of such assessment, which must be approved by the Executive Committee before becoming effective." Seconded, by Elise Shover; carried.

F. A. Collatz moved that Article VI, Section 5(*b*), be changed to read as follows: "Honorary and Corporation members shall be entitled to receive the regular publications of the Association and the privileges of membership in a local section." Seconded, by R. L. Frye; carried.

William R. Green moved that no changes be made to the present Article VII, Section 1(*b*). Seconded, by P. E. Minton; carried.

R. L. Frye moved that no changes be made to Article VIII, Section 2. Seconded, by C. B. Kress; carried.

L. D. Whiting moved that the word "Secretary-Treasurer" in Article VIII, Section 2, be changed to read "Secretary." Seconded, by Pearl Brown; carried.

L. W. Francis moved that the word "Secretary-Treasurer" be changed to read "Treasurer" wherever it appears throughout the Constitution. Seconded, by H. G. Walter; carried.

Washington Platt moved that an honorarium of one hundred dollars be given Secretary-Treasurer M. D. Mize in appreciation of the service he has rendered the Association during the past year. Seconded, by M. A. Gray; carried.

Report of Committee on Osborne Medal Award

Paul Logue, *Chairman*

The Committee on Osborne Medal Award has carefully reviewed the contributions to the science of cereal chemistry of each of the men whose names have been presented either from within the committee or from the Association at large.

The weighing and balancing of the relative merits of fundamental contributions to the knowledge of starch and proteins, for example, compared with the more practical contributions of a commercial aspect, has involved much thought and deliberation on the part of the committee.

The selection has been difficult, not through any lack of deserving men, but rather through the abundance of valuable technical contributions to cereal chemistry by several men.

The Committee respectfully submits the name of Dr. M. J. Blish as the next recipient of the Osborne Medal Award.

Paul Logue moved that this report be accepted. Seconded, by E. E. Smith; carried.

Meeting adjourned at 4:15 p.m.

Saturday, June 8

Meeting called to order, 8:45 a.m. by President, Mary M. Brooke.

Report of the Publicity Committee

H. W. Putnam, *Chairman*

Messrs. Frye, Gray, Haley, Marx, Stateler, and Putnam, members of the Publicity Committee for 1934–35, offer the following report of their activities in keeping the constructive work of the Association before the public.

During the year, the exceptionally fine local section programs, in which speakers of note were featured, have been the basis for effective publicity contained in seventy releases to the trade press. Forty-one interested publishers have asked to be on our mailing list.

This increasing interest on the part of the food trade press has been due to the broadened interests of the Association.

This group of publishers may be roughly divided as follows: five, milling; seven, baking; nine, brewing; three, pure science; three, popular science; six, trade papers; and eight, of general interest.

In addition to the news releases, fourteen communications were sent to the various section officers and others to convey pertinent news items or to help coordinate certain activities of the Association. Chairmen and secretaries have cooperated by promptly furnishing news items and by exchanging information on their activities with their fellow chairmen.

Advance copies of the abstracts of papers given at the Annual Meeting have been of assistance in preparing running news stories.

The "Memo-Card" containing names and addresses of officers, section chairmen, and past presidents, together with data on Section Meetings, and an announcement of the Denver meeting was mailed to all members. This is the fourth successive year for what has now become an institution with the Association.

The Committee has acted as a clearing house for the moving picture films which have been taken at past annual meetings and during the year has loaned these for use at several section meetings.

As mentioned in the report last year, the Committee is still aware of certain definite shortcomings in connection with its attempt to reach the consuming public with popular and informative interpretations of cereal chemistry. For this, we must still depend upon the energy and initiative of our membership in taking advantage of all opportunities for presenting such information through speaking and writing whenever the opportunity is available. It has been suggested that a properly written series of articles could be given wide publicity through the smaller newspapers. Certainly there is ample room for every member of the Association to speak for himself and his profession in making more effective the work of future Publicity Committees.

H. W. Putnam moved that this report be accepted. Seconded, by H. H. Johnson; carried.

Report of Employment Committee

C. B. Morison, *Chairman*

Letters, correspondence from June 4, 1934 to June 1, 1935	98
Positions filled	5
Employers requesting assistance	6
Names registered 1934–1935	12
Names registered 1933–1934	19
All names (two years)	31

237 applicants have filed names with the Chairman's office.

C. B. Morison moved that this report be accepted. Seconded, by H. G. Walter; carried.

Report of the Committee on Definition of Moisture Basis for Laboratory Reports

R. K. Durham, *Chairman*

This committee was created during the annual A.A.C.C. meeting in 1932, to consult and co-operate with the Millers' National Federation and others, in establishing moisture basis for reporting chemical constituents of wheat, flour, and feeds.

At the same meeting the A.A.C.C. methods committee had recommended, "that a definite moisture basis be adopted for reporting chemical constituents of wheat, flour, and feeds." That recommendation together with the name which was later given to this committee might be construed to mean that its members are assigned the duty of selecting the basis as suggested by the Methods Committee. A different interpretation, however, has been made by the members of your committee on Definition of Moisture Basis for Laboratory Reports. We feel that the motion creating this committee together with the discussion which followed, rather clearly limited our committee to *co-operation* with other organizations interested in moisture bases.

When this committee originated there appeared to be considerable activity along these lines. The Millers' National Federation and the Southwestern Millers' League had adopted resolutions requesting various wheat testing agencies to report wheat protein on a 15.00% moisture basis. The Kansas State Board of Agriculture was seriously considering the problem of reporting analysis of wheat mill feeds on a fixed moisture basis. Furthermore, some of the cereal chemists were inclined to doubt the wisdom of reporting flour analysis on a 15.00% basis.

But since that time there has been a gradually increasing lack of interest and activity. The Millers' National Federation and the Southwestern Millers' League are not disposed to follow up their resolutions asking that wheat protein be reported on a 15.00% moisture basis. And if they were, it is quite probable that considerable resistance would be encountered, because, while the officials of the Kansas State Grain Inspection Department have indicated that some basis might be acceptable to them, they certainly would not approve of 15.00%.

The feed situation has likewise been shelved. Following a series of hearings on the subject of moisture basis for feeds, which culminated in a request by the millers that a 15.00% basis be adopted, the Kansas State Board of Agriculture stated that the proposed basis was regarded as unreasonably high and that it would operate to the disadvantage of the consumer.

The question of a basis for conversion of flour analysis has received little or no attention by other organizations, hence there has been no co-operative discussion.

It now appears that the immediate need for co-operative thought and action as it existed in 1932 has almost disappeared.

So long as this committee is in existence it may impede whatever progress might be contemplated in the deliberation of the Methods Committee's recommendations that a definite moisture basis be adopted.

It is, therefore, recommended that this committee be discharged.

R. K. Durham moved that this report be accepted. Seconded, by William R. Green; carried.

The members of the Local Arrangements Committee, Mrs. H. D. Liggett, H. D. Liggett, H. R. Fisher, W. C. Rohrbaugh, E. G. Holden, H. M. Lair, C. C. Armuth, H. W. Putnam, Clarence Stull, and H. J. Fine, were brought before the convention and President Mary M. Brooke expressed the appreciation of the Association for the work accomplished by this Committee, and extended the thanks of all members present for the entertaining visit enjoyed by all during the meeting. Especially was Mrs. Liggett commended upon the entertainment furnished the ladies.

Washington Platt moved that a vote of thanks expressing the appreciation of each member present be given the Colorado Mill and Elevator Company for their assistance with the convention. Seconded; carried.

The newly elected officers were officially installed for the ensuing year, and the meeting was placed in charge of the new President, Washington Platt.

President Platt appointed the following committees for the year:

Executive Committee

H. D. Liggitt, *Chairman*
Washington Platt
Mary M. Brooke
R. C. Sherwood
Geo. Garnatz

Convention Program Committee

M. J. Blish, *Chairman*
F. L. Dunlap
C. O. Swanson
R. C. Sherwood
H. W. Rohde

Dallas Local Arrangements Committee

Rolfe L. Frye, *Chairman*
G. E. Findley
Helman Rosenthal
F. A. Bleger

Membership Committee

Elsie Singruen, *Chairman*
Chairmen of the twelve local sections.
Bert Ingels, Member at large.

Committee on Methods of Analysis

Betty Sullivan, *Chairman*
C. F. Davis
E. G. Bayfield
A. E. Treloar
R. M. Sandstedt
Oscar Skovholt
W. L. Heald

Committee on Standardization of Laboratory Baking

C. O. Swanson, *Chairman*
W. F. Geddes
C. N. Frey
M. J. Blish
R. K. Larmour
C. F. Davis

Malt Analysis Standardization Committee
(Please note change of name)

D. A. Coleman, *Chairman*
J. D. Veron
Elsie Singruen
H. W. Rohde
H. C. Gore
L. E. Leatherock

Committee on Cake Baking Tests
Membership to be announced.

Committee on Testing Biscuit and Cracker Flours

Walter Reiman, *Chairman*
E. L. Ulrey
Pearl Brown
C. O. Oppen
Jan Micka
J. C. Lankenau
S. M. Jackson

Committee on Methods of Testing Self-Rising Flours

H. G. Walter, *Chairman*
H. V. Moss
R. A. Barackman
L. D. Whiting

Committee on Testing Pie Flours

C. B. Kress, *Chairman*
L. Bisno
P. O. Hastings
W. K. Marshall
C. C. Fifield

Committee on Testing Rye Flours

L. H. Bailey, *Chairman*
J. T. Flohil
T. W. Sanford
L. W. Haas
G. Moen

Committee on Definitions of Technical Terms

Membership to be announced.

Committee on Employment

C. B. Morison, *Chairman*
C. H. Bailey
H. W. Putnam
C. A. Glabau
N. L. Gregory

Publicity Committee

T. R. West, *Chairman*
R. L. Frye
H. W. Putnam
V. E. Marx
E. S. Stateler
C. A. Glabau
W. A. Richards

History Committee

R. J. Clark, *Chairman*
L. R. Olsen
R. W. Mitchell

Committee on Osborne Medal Award

Paul Logue, *Chairman*
C. L. Alsberg
E. E. Smith
C. G. Ferrari
C. H. Bailey

Inter-Relations Committee

Membership to be announced.

Report of the Special Auditing Committee

J. A. LeClerc and L. H. Bailey

The Special Auditing Committee has examined the books of the Managing Editor for the calendar year 1934 and found the same to be correct.

C. C. Fifield moved that this report be accepted. Seconded, by Mary M. Brooke; carried.

C. H. Bailey was placed in charge of the meeting as Chairman of the Morning Program.

Report of the Committee on Definitions of Technical Terms

C. H. Bailey, *Chairman;* read by Q. Landis, *Secretary*

While the committee has benefited greatly from the discussion at Toronto and by individuals elsewhere of the terms proposed for consideration by the preceding committee, it is believed that the time is not yet ripe for definite action upon these proposals. The committee therefore recommends that no action be taken upon the proposed definitions, but that they be earnestly studied and considered in their full connotation by the members of the Association during the coming year. Minor changes in some of the proposed definitions as published in the late News Letter have been made.

In view of the dual meaning now ascribed to "stability" it has been deleted from the proposals for the time being, but it is recommended nevertheless that the members of the Association keep the term under consideration. As the report of the preceding committee indicates, the greatest difficulty was encountered with strength. It was decided to present the opinions of each member of the committee separately for your consideration and to recommend that final judgment be suspended for the moment, hoping that as further data are obtained by the Baking Test Committee a more logical and precise definition may emerge from the information so obtained.

The proposals are as follows:

Weight of flour specified in all definitions shall be the equivalent of the quantity stated at a definite moisture content of 13.5%.

(*a*) *Standard loaf* is one which possesses an adequate combination of volume,

textural and external characteristics and desirable palatability. This definition will be subject to amplification and refinement as baking test methods are developed and standardized. In the meantime it should be described as fully as possible by the experimenter.

(*b*) *Response* is the reaction of a dough to a known and specific stimulus, substance, or conditions. It may be positive (plus differences), neutral (zero differences), or negative (minus differences). When not otherwise qualified, response shall be understood to represent the difference in loaf volume in cubic centimeters per 100 grams of flour. Other responses may be reported, such as response in terms of texture. Unless otherwise stated, favorable responses shall be recorded as positive, unfavorable as negative.

(*c*) *Tolerance* is the amount of any single stimulus, substance or condition which registers a neutral response in a dough, this amount being measured from the point at which a standard loaf first appears to the point at which the loaf characteristics become substandard. It represents the range over which positive and/or neutral response is registered. Amongst other variables, tolerance may be a function of time.

(*d*) *Strength*

1. Proposed by Q. Landis.

"Strength is the inherent ability of a flour to produce loaves of leavened bread, provided the gas production factor is adequate, under scientifically controlled baking conditions (Humphries, Kent-Jones, Mangels, and Sanderson) and may be measured numerically by protein content (Blish), or loaf volume at maximum responses (Kent-Jones and Geddes)."

2. Proposed by C. B. Morison.

"Strength is the ability of a sound flour to produce a loaf of bread of standard external and internal characteristics. Strength is dependent upon the physico-chemical composition and properties of the flour and also upon the baking procedure employed. Protein content may be regarded as an index of flour strength, provided that other influencing factors or conditions are not excluded."

3. Proposed by M. J. Blish and M. A. Gray.

"Strength is that property of flour that, when subjected to established and accepted commercial baking procedure, produces bread of satisfactory symmetry, size, texture, and grain and may be measured numerically by protein content."

Quick Landis moved that this report be accepted. Seconded, by M. C. Markley; carried.

Paper—"Varietal Variation in Durum Wheat Starches," by C. E. Mangels, read by C. B. Morison.

Paper—"Bread Judging Activities, New York Section," by B. D. Ingels.

Paper—"Proteolytic Enzymes of Flour," by A. K. Balls and W. S. Hale, read by A. K. Balls of U. S. Department of Agriculture.

Paper—"Diastatic Activity in Doughs and Suspensions. Modification with Active Supplements," by Quick Landis.

F. L. Dunlap was called before the convention by Mary M. Brooke, who extended the thanks of the Association for his work and co-operation in arranging the excellent program enjoyed by all during this convention.

Paper—"Diastatic Properties of Mill Streams," by Quick Landis and H. K. Steele, read by Quick Landis.

Paper—"Studies of Dough Formation," by M. C. Markley, F. L. Harrington and C. H. Bailey, read by M. C. Markley.

Paper—"Measurement of Respiration in Flour," by E. B. Working.

Paper—"New Developments in Cereal Chemical Research," by C. L. Brooke, read by M. C. Markley.

President Washington Platt resumed charge of the meeting.

Report of the Committee on Resolutions

C. B. Morison, *Chairman*

Whereas, The American Association of Cereal Chemists has just completed another annual meeting and its success has been due largely to the painstaking interest and splendid service of all our officers and committees,

Therefore, be it resolved, that the appreciation of this Association be expressed to Mrs. Mary M. Brooke, President; Washington Platt, Vice-President;

M. D. Mize, Secretary-Treasurer; F. L. Dunlap, Chairman of the Program Committee; H. D. Liggitt, Chairman, Local Arrangements Committee, and Mrs. H. D. Liggitt, Chairman, Ladies Entertainment Committee.

Be it further resolved, in accordance with precedent, that our retiring President be presented with a suitable testimonial in permanent form as a small token of our individual and collective esteem.

Be it further resolved, that we express our appreciation of the services of Dr. D. A. Coleman, Editor-in-Chief, CEREAL CHEMISTRY, and C. C. Fifield, Managing Editor, CEREAL CHEMISTRY, in maintaining the high value of our journal CEREAL CHEMISTRY.

Be it further resolved, that we express our thanks to the Reverend A. W. Liggett of Denver, Colorado, for the invocation so reverently presented at the opening session of the meeting on June 4th.

Be it further resolved, that we express our sincere thanks to Mr. F. H. McInroy, who so graciously welcomed us to the City of Denver on behalf of the Governor of the State.

Be it further resolved, that we express our appreciation of the fine co-operation of Dr. James T. Jardine, Chief, Office of Experiment Stations, United States Department of Agriculture, in addressing our Association on the cereal work of the Agricultural Experiment Stations.

Be it further resolved, that the appreciation of the Association be expressed to Mr. Gordon H. Darnell, a Past President of the American Society of Bakery Engineers, for his excellent co-operation in bringing the problems of the baker's production manager to our attention for study, review, and joint educational effort in the formation of a better common understanding of mutual problems.

Be it further resolved, that we extend our heartiest appreciation to Captain Pitt and Sergeant Bemis of the Denver Police, and Fred Curtis, D. Piccone, W. E. Sager, and Walter Haskell of the State Police, for the efficient way in which they supervised the auto tour to Golden and Bergen Park and thus reduced the hazard of accident to ultimate zero.

Be it further resolved, that we extend our thanks to The Victor Chemical Company, Standard Brands, Inc., Wesson Oil Company, Denver Fire Clay Company, Chemical Sales Company, Laboratory Construction Company, Wallace & Tiernan Company, Lever Brothers, and Bert Minton for their generous contribution of the golf prizes.

Be it further resolved, that we extend our thanks to the Provident Chemical Works for their interest and assistance in conducting the Ladies Bridge Party.

Be it further resolved, that we express our thanks to The Merchants Biscuit Company, The Sperry Flour Company, Chemical Sales Company, The Mine & Smelter Supply Company, and the Denver Fire Clay Company for their very generous use of automobiles for the tour to Golden and Bergen Park.

Be it further resolved, that we extend our thanks to The Gates Rubber Company, The Filtration Plant, The Merchants Biscuit Company for the most interesting and educational inspection trips so courteously arranged for our members.

Be it further resolved, that we extend our hearty thanks to Mr. Adolph Coors for the excellent luncheon and inspection trip throughout the Coors plants. Mr. Coors' hospitality is well known to American chemists, everywhere, and it is a privilege for our Association to add our own sentiments to those of many others in all fields of chemistry.

Be it further resolved, that the need of more fundamental scientific research on the problems of cereals and their products in the interest of the economics of agriculture, the public welfare, the improvement of present quality, and their better utilization and distribution is recognized by the American Association of Cereal Chemists as of the highest importance to future national well being.

Therefore, we respectfully suggest that the United States Department of Agriculture may well consider further support and extension of fundamental scientific research as a necessary step in the fulfillment of these objectives.

Be it further resolved, that we express our thanks to the United States Department of Agriculture for its fine co-operation in permitting several of its research workers to participate in the program of our annual meeting.

Be it further resolved, that we extend our thanks to the Denver Fire Clay Company for the use of their projection lantern throughout the meetings.

Be it further resolved, that we express our thanks to the Colorado Mill and Elevator Company and its officers and employees for their wholehearted efforts in making the Denver meeting a real and memorable success.

Be it further resolved, that we extend our appreciation to the Denver Travel Bureau for their very gracious and excellent manner of conducting the registration.

Be it further resolved, that we extend our thanks to Mr. M. L. Tracy, Manager of the Brown Palace Hotel, for his wholehearted co-operation in conducting the convention activities.

Whereas, during the year three of our members were deceased,

Be it resolved, that we express our deep sympathy at the passing of T. A. Gierahn, G. E. Howe, and M. B. Graff.

Be it further resolved, that we express our sincere sympathies to the families of these deceased members.

C. B. Morison moved that this report be accepted. Seconded, by Pearl Brown; carried.

Convention adjourned by President Washington Platt.

REGISTRATION OF CONVENTION, DENVER, COLORADO

June 4–8, 1935

Members

F. W. Albro
Geo. L. Alexander
A. A. Andre
Chas. C. Armuth
C. H. Bailey
Dr. J. C. Baker
R. A. Barackman
J. Glenn Blanch
F. A. Bleger
M. J. Blish
L. J. Bohn
R. T. Bohn
G. S. Bratton
Mary M. Brooke
Pearl Brown
H. J. Brownlee
W. E. Brownlee
T. E. Carl
Lee Clark
D. A. Coleman
F. A. Collatz
A. E. Curtis
J. R. Davies
C. F. Davis
C. E. Day
Joseph DeHaan
F. L. Dunlap
R. K. Durham
C. C. Fifield
G. E. Findley
Herman Fine
H. R. Fisher
V. E. Fisher
John T. Flohil
L. W. Francis
J. Freilich
C. N. Frey
R. L. Frye
W. F. Geddes
C. A. Glabau
Edw. Gookins
M. A. Gray
Wm. R. Green
Newman L. Gregory
Frank L. Gunderson
L. W. Haas
Wm. L. Haley
C. G. Harrel
W. L. Heald
E. G. Holden
Bert D. Ingels
L. E. Jackson
Stanley M. Jackson
H. H. Johnson
R. M. Knudson
C. B. Kress
H. M. Lair
A. L. Lancaster
Quick Landis
L. E. Leatherock
H. D. Liggitt
Paul Logue
Harry J. Loving
J. M. Lugenbeel
Fred J. Lumsden
C. H. MacIntosh
D. A. MacTavish
B. A. McClellan
L. H. McLaren
D. A. Magraw
Max C. Markley
Victor E. Marx
Louis A. Mennel
Paul P. Merritt
P. E. Minton
H. S. Mitchell
R. W. Mitchell
M. D. Mize
G. Moen
J. W. Montzheimer
C. B. Morison
H. V. Moss
Leslie Olsen
Clarence O. Oppen
P. R. Pitts
W. Platt
R. B. Potts
H. W. Putnam
W. L. Rainey
J. W. Read
W. C. Rohrbaugh
Perie Rumold
J. W. Schlesinger
J. A. Shellenberger
R. C. Sherwood
Elise C. Shover
O. P. Skaer
Oscar Skovholt
Edw. E. Smith
George Stadler
W. E. Stokes
Clarence Stull
Betty Sullivan
W. B. Thrasher
N. F. True
C. G. Vaupel
E. A. Vaupel
James D. Veron
C. C. Walker
H. G. Walter
L. D. Whiting
H. W. Winkler
Earl B. Working

Visitors and Guests

Mrs. A. A. Andre
Mrs. Chas. C. Armuth
Mrs. R. A. Barackman
Mrs. R. T. Bohn
Mrs. G. S. Bratton
Mrs. W. E. Brownlee
Miss Charlotte Chatfield
Mrs. F. A. Collatz and Son
Mrs. A. E. Curtis
Mrs. C. F. Davis
Miss Marcia Davis
Mrs. C. E. Day
Mrs. Joseph DeHaan
Miss Ruby DeHaan
Mrs. C. C. Fifield
Mrs. G. E. Findley and Sons
Mrs. Herman Fine
Mrs. H. R. Fisher
Mrs. John T. Flohil
Mrs. L. W. Francis
Mrs. R. L. Frye
Mrs. W. F. Geddes
Mrs. Edw. Gookins
Mrs. M. A. Gray
Mrs. Wm. R. Green
Mrs. Frank L. Gunderson
Mrs. L. W. Haas
Mrs. C. G. Harrel
Mrs. W. L. Heald
Mrs. E. G. Holden
Mrs. L. E. Jackson
Mrs. H. M. Lair
Mrs. A. L. Lancaster
Mrs. H. D. Liggitt
Mrs. Harry J. Loving
Mrs. J. M. Lugenbeel
Miss Marjorie Lugenbeel
Mrs. C. H. MacIntosh
Mrs. B. A. McClellan
Mrs. L. H. McLaren
Mrs. D. A. Magraw
Mrs. Joe Medina
Mrs. Paul P. Merritt
Mrs. P. E. Minton
Mrs. G. Moen
Mrs. Leslie Olsen
Mrs. Clarence O. Oppen
Mrs. Ralph B. Potts
Mrs. H. W. Putnam
Mrs. O. H. Raschke
Mrs. W. C. Rohrbaugh
Mrs. A. H. Rosseau
Mrs. Perie Rumold
Mrs. J. W. Schlesinger
Mrs. R. C. Sherwood
Miss Elsie Singruen
Mrs. Edw. E. Smith
Mrs. Geo. Stadler
Mrs. W. E. Stokes
Mrs. Al Thornton
Mrs. W. B. Thrasher
Mrs. E. Lohmueller
Mrs. C. G. Vaupel
Mrs. E. A. Vaupel
Mrs. Jas. D. Veron
Mrs. H. W. Winkler
Mrs. Earl B. Working
A. K. Balls
M. A. Barmore
Henry R. Bicknell
C. R. Cedarberg
Gordon H. Darnell
Lorenzo DeHaan
Phil Goldfisch
Wm. Heald
Henry Jardine
D. B. Jones
Henry Jungerman
J. C. Kephart
Arthur J. King
J. T. Mannion
Joe Medina
Emil Munz
Harry Obermeyer
L. W. Pingree
A. Pitann
O. H. Raschke
Armand H. Rosseau
E. N. Ryan
C. H. Stafford
Al Thornton
Mr. Walker

PRESIDENTIAL MESSAGE

MARY M. BROOKE

Puritan Mills, Chicago, Illinois

(Read at the Annual Meeting, Denver, June 1935)

It is usually customary for the president of this Association to make a report of the stewardship of their term of office and then to discourse briefly or at length upon some fatherly or, in this case, motherly advice upon the activities of the Association, past, present, or future. Nature seems to have endowed me with all of the prerequisites of maternalsim, including face, figure, and gray hairs. Added to this are years of life, too many to mention, and years of service, in this particular field of endeavor, numbering almost twenty.

It would be extremely incongruous and certainly an inexcusable faux pas on my part to attempt any learned and scientific discourse in this address. I am not, nor ever will be a great scientist.

So, Mother Brooke, the chemist, in lieu of a scientific gem, is going to endeavor in an admonishing way, with the excuses offered, to presume to scatter some pseudo pearls of wisdom and advice. I can assure you that this is going to be done in a very humble and deprecating manner, realizing full well that the Association and its ideals are greater than any individual and their ideas, no matter whether that person holds the highest office or is merely one who sends in their dues about the first of the year.

First of all, we will endeavor to make a report of our stewardship, with interspersions. The usual committee appointments were made at the Toronto meeting. Probably, as a result of cowardice, the number of committees remained intact. I am a great believer of committee work, in that I believe it serves the useful purpose of getting work done systematically and, secondly, that it gives more members a chance to enter into the activities of the Association. But even I, in my enthusiasm, feel that it can be and probably is being overdone. The programming of these reports alone is a great task. And yet, if the work is done, it should be acknowledged and discussed. Our present set-up of committees is like Topsy, "it just grew." A revision of these, as well as the constitution, is advised. It seems to me that the work of some of these committees could better be done by individuals who are probably quite vitally interested. I would like to have each committee chairman

include in the report an expression as to the future continuation of his or her committee either in its present form or in a different form.

One new committee was brought into existence and that was the Committee on Methods of Brewing and Malting Control. After a careful survey, it was found that the interests of the Association were much more diversified than was realized. The purposes of the Association as stated in the constitution provide for all cereal work. We have a great many members who are interested in this problem. We have a great number of potential members interested in these problems. This committee was formed, merely, to start this work and interest. It has been successful under the direction of Dr. Coleman and highly justifies its existence.

The Executive Committee has attempted a revision of the constitution. This move was decided upon in Toronto. The details were worked out later. The salient points in this revision are the Financial Advisory Committee, as proposed by Mr. Platt, the separation of the offices of Secretary and Treasurer, as proposed by Mr. Mize, and the shortening of the term of publication of revisions of the constitution, as proposed by Dr. Sherwood. The remainder of the proposals are detailed revisions to clarify the meanings of sections and articles.

There was one proposed revision not published. This revision has been broached both formally and informally from time to time and I believe should be brought up by the membership at the discussion on Friday afternoon. That proposal is to provide for balloting for officers by mail rather than at the convention. There are a great many arguments in favor of this type of election, the principal one being the small percentage of the membership who can participate in convention balloting versus the entire participation by mail. Again, I am going to use the word cowardice in describing our attitude in at least not giving this serious consideration and discussion.

The most important change of the constitution, as proposed, is the separation of the secretary-treasurer's office as proposed by Mize. This proposal has been the subject of considerable controversy and no agreement. With the continued growth of the Association in membership and activities, the burden of detail work and major problems has tremendously increased. This, in turn, has placed more duties upon the officers to the extent that it has become burdensome to the individual and to their employers to carry on. It is the thought that in the separation of these offices and in the creation of a fourth, a definite division of work might be made and relief both of the treasurer's and presidential offices may be accomplished. Also that closer contact may be maintained with local sections and that more necessary work may be accomplished beneficial to the best interests of the Association.

You have all read the printed treasurer's report and know the Association to be in good condition financially with a surplus at the end of the year. Our membership has grown in spite of the troublesome times and we have a net gain of 27 members. According to plans made during Dr. Sherwood's term of office, the methods committee has prepared and published during the year the book entitled "Cereal Methods." This book was sold to the membership at the lowest possible price to cover costs. The number of orders, which were very gratifying, make the printing of the book a solvent adventure. The history of the association is now in your hands. This history was prepared by Ralph Mitchell. The usual number of News Letters have been published. This means of communication is becoming more valuable all the time, although the volume of work involved in their publication is by no means small. It is the one means that the officers have at their disposal of communication with the membership during the year.

There is no need of covering the activities of CEREAL CHEMISTRY in this address. You have the published account of its finances along with the six numbers published during the year. In other words, they have written their own report. Opportunity is to be given during the program for Dr. Coleman to informally discuss with the members, the policies of the editorial board in the choice of the papers for publication.

So much for the accounting. In carrying out these projects it has been the wish of your president to advisedly comply with the wishes of the majority of the membership. I only hope that I have attained that goal and have earned for myself a place with my illustrious predecessors.

From this point, I am going to choose as my text, Article II of your constitution. It is getting to be a habit of mine to use this article as a text. I am trying to make it as well known to cereal chemists and their friends and employers as the famous Article X of the League of Nations. In so doing, I am acting advisedly as I feel at times that we as members are losing sight of our lodestar and are letting lesser stars confuse us and lead us off the chosen course. This article was written after serious and learned consideration. The words and meaning are well chosen and if religiously followed will lead to a successful accomplishment of the chosen aims and purposes. This article definitely states our purposes as those of a scientific society founded for the purpose of carrying on co-operative scientific work, encouraging scientific work, and the maintaining of high professional standards. It says nothing about profit, commercialism, and personal aggrandizement, which is as it should be. In short, this is proposed to be a body of unselfish, highly idealized scientists, and not a trade or political association. We are not to be involved in any of Amos and Andy's "corpulation" activities.

Our founders and originators did much to insure that these ideals were inculcated in the infant association. If we go back into our history we find many wonderful examples of men who gave unstintingly of their time and ability in order that success, both from the standpoint of organization and finances might be attained. We as members, at the present time, can only show our appreciation of these efforts and unselfishness by following their precepts of high ideals and wide vision.

How may we as worthy members show this appreciation? First of all, by judging a member, irregardless of the purpose of passing this judgment, on his merits and ability, first as a scientist and secondly as a man, not on his business connections and their relations to ours, not on the specific type of work, which he or she happens to be doing at the moment, or where he may live. Time, after all, may change any and all of these things. True science does not always hold a doctorate, although we pass in reverence to the one who does, for all it implies. True science may be found in the humblest and least assuming of us. It is for us to discover, to recognize, and to see that full credit is given where it belongs. The practical application of this idealism is the choice of officers, committee chairmen, and speakers or any other means which we have of showing our collective recognition of that person.

Our second means of following our precepts is by holding the business organization and business relations of the Association on a high and ethical plane. As was stated before, we are not organized for profit, yet we must remain solvent. We are at the present time solvent with a goodly surplus. So there is no reason for assuming any attitude that might be judged commercial. We wish to encourage membership, not from the standpoint of membership fees, but from the standpoint of the interest which that prospective member has in our association and from the interest which we as scientists may have in that member and the work which we may as an association encourage that member to undertake. This may sound selfish, but if we give recognition to that person it is also altruistic. The benefits should be mutual. We wish to encourage the membership of the fellow who is off the beaten path of communication and needs our association as the only means of contact with developments in his work.

We should not either hope for or encourage donations or contributions of time, money, or anything else of intrinsic value from our employers or interested firms, except for scientific promotion. What we wish from these people is recognition of the value of cereal chemistry and its application to their problems, of our value as an association in the encouragement of this work, and of our personal value as an interpreter. That is the most valuable thing that they can give to us and which we can hope to attain.

Admittedly our goal is high but no one ever attained success without a goal. Our goal is one of prestige and the highest scientific recognition. Let us not do anything individually or collectively which will lower our prestige in the eyes of anyone. Having gained prestige, scientific recognition must of necessity follow.

This goal being reached, we have then and only then fulfilled the purposes of our Association, the now famous Article II, to wit:

Article II

Purposes of the Association

The purposes of this Association are (1) the encouragement and advancement of scientific and technical research in cereals and their products, particularly in milling and baking, but including also other industries in which cereals and their products are utilized, (2) the study of analytical methods used in cereal chemistry and the development and adoption of uniform (or standard) methods of examination and analysis, (3) the promotion of the spirit of scientific co-operation among all workers in the field of cereal knowledge, (4) the maintenance of high professional standards in the Association as conditions of membership and, (5) to encourage a more general recognition of the chemist and biologist as essential factors in the development of the cereal industries. In accordance with these purposes this Association shall conduct a journal in which contributions to the scientific knowledge of cereals, their products and technical application shall be published for the encouragement and advancement of the science. It shall hold meetings for the discussion of cereal knowledge and the promotion of research and technical co-operation among its members.